PHYSIOLOGICAL REVIEWS

VOL. XIV JULY, 1934 No. 3

# THE INFLUENCE OF NUTRITION UPON RESISTANCE TO INFECTION

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The possibility that diet may have some influence upon the incidence, course, and final outcome of infection, is a comparatively recent idea. Since 1900 the idea has gained ground, and quite a body of work has appeared in the literature. The task of reviewing it is not easy for several reasons: in many cases the results are contradictory, in others they may be difficult of interpretation because of many variables. At best the literature is a scattered one. In considering the actual infection, the author has confined himself to infections of bacterial origin, and has not included, for lack of space, much excellent and suggestive work on infections of protozoan and metazoan origin.

In general one may say that the work in this field is in its infancy, but that there is much suggestive work that merits further study.

*Vitamin B complex.* Petragnani (1921) claimed that pigeons, fed on polished rice, lose their immunity, both natural and acquired, to anthrax, even before symptoms of polyneuritis develop. Corda (1923) believes that this loss of immunity may not be due to deficiency of vitamin B, but may in part be ascribed to underfeeding. Healthy adult pigeons, starved four days, or fed only 10 grams fresh asparagus tips for four days, die within two days after receiving injections of anthrax cultures—i.e., as promptly as do pigeons with polyneuritis. No attention was given to the temperature of the animals, although Pasteur had clearly shown that chilling abolishes the natural resistance of the chicken to anthrax. G. M. Finlay (1923) was able to show that normal animals, whose body temperature is lowered by pyramidon, or in the course of vitamin B deficiency, invariably die if inoculated with pneumococcus, B. coli, or B. enteritidis; whereas they nearly always survive these infec-

tions if the body temperature is normal (41° C.), whether they are suffering from deficiency of vitamin B or not. According to Verder (1928), B. enteritidis, fed to rats, does not pass through the intestinal wall in animals unless deprived of the vitamin B complex of yeast.

Among the investigators who believe that vitamin B may increase resistance, should be mentioned W. B. Rose (1928), who reports that the natural immunity of the dog to infection with B. welchii disappears when that animal is deprived of vitamin B; and that in a number of cases, dogs with (gas bacillus) bacteremia, were cured by injections of "B" concentrate. Since dogs deprived only partially of this vitamin, and gaining in weight, were also susceptible, the lack of resistance can not be due to inanition. McCarrison (1919) had observed spontaneous bacteremia due to B suipestifer in birds with polyneuritis. Barlow (1930) shows that this phenomenon is not dependent solely upon the anemia which accompanies severe "rice disease," but may be observed in the absence of anemia in mild deficiency; and that it can be abolished by feeding yeast. Unfortunately, he does not clearly establish the type of bacteria, the portal of entry or their pathological significance. In an extensive study of dietary factors and infection, Ross and Robertson (1932) report that rats, deprived of the vitamin B complex, are much more susceptible than are controls, to oral infection with Salmonella muriotitis. In these experiments, the deficient diet was fed to rats aged four weeks; the infection was induced 1 to 8 days afterwards; and the rats which died succumbed 1 to 24 days thereafter. Inasmuch as the average life of such rats, if uninfected, is only 28 days (Sherman, 1919) it seems possible that the somewhat earlier mortality observed is due merely to an already existing weakness.

It has even been shown by Reiner and Paton (1932) that rats suffering from deficiency of the B complex and infected with Trypanosoma equiperdum live longer than infected controls.

Taken as a whole, these experiments give somewhat conflicting results. It would seem, at any rate, that in this type of experiment, the factor of body temperature must be controlled, in order to gauge accurately the effect of vitamin B deficiency on immunity. Also the possibility of inanition, or, at least, the lessened food consumption of the sick animal, as a factor contributory to the results, must be taken into account.

*Vitamin C.* Although the cure of scurvy by diet was known to navigators four centuries ago, the epidemic nature of the disease suggested a bacterial origin to many investigators in the era of Pasteur. There can be no doubt that in man, and in experimental animals, infections of

various kinds may cause a latent scurvy to develop into the outspoken disease. Jackson and Moody (1916) cultivated from organs of scorbutic guinea pigs a green producing streptococcus, which, injected into animals fed on a scurvy producing diet, led to the rapid progression of scorbutic lesions and death. It had little effect on normal animals. This work is an early example of the use of an organism peculiarly pathogenic for the animal studied, during the development of a deficiency disease. It is likely that in guinea pigs, suffering from entire deficiency of vitamin C, and moribund in consequence, almost any form of injury will bring about death. A partial deficiency in the experimental animal might give better results. Findlay (1923) therefore produced a type of chronic scurvy, by giving an inadequate amount of the anti-scorbutic factor, 2 cc. of orange-juice, every third day. Although the guinea pigs were in good general condition, they were found to be very much more susceptible than controls to the intraperitoneal injection of pneumococci, S. aureus, S. hemolyticus, and B. coli. Other authors have shown that spontaneous fatal infections are also much more likely to occur in scorbutic animals. Nicolle (1912) called attention to the frequency of pneumonia which occurred in guinea pigs which were fed on poor diets. Theobald Smith (1913) observed that epidemic pneumonia promptly ceased in his colonies of guinea pigs, when fresh green fodder was provided. The beneficial factor was not identified, and no evidence of latent scurvy was mentioned in these observations. In a study of 400 animals, Wámoscher (1927) showed clearly that subacute scurvy in guinea pigs predisposes to spontaneous pneumonia. The course of the illness was followed by Röntgen-ray. The facts that many of the organisms isolated were not pathogenic for normal guinea pigs, and that cure sometimes followed the use of large quantities of orange-juice, are of great significance. In attempting to induce infection of scorbutic guinea pigs by inhalation of droplets containing B. suipestifer and pneumococcus, Schmidt-Weyland and Költsch (1927–28) were not always invariably successful. They clearly proved that infection does not take place until the resistance of the animal has fallen very considerably. The scorbutic animals, which were already carriers, as shown previously by nasal cultures, succumbed more frequently than did those in whom the disease was not so far advanced. Injury of the mucous membrane by silver nitrate or by mustard oil, will frequently bring about loss of resistance in normal animals. Scorbutic guinea pigs, infected with Streptococcus hemolyticus, develop lesions very similar to those of rheumatic fever in man (Rinehart, Connor and Mettier, 1934). Many in-

vestigations have been made of tuberculosis in scorbutic animals; see section on *Tuberculosis.*

There can be no doubt that in the guinea pig, scurvy lowers resistance to certain types of infection. The mechanism involved will be considered later.

*Vitamin D.* The supposed lack of resistance to infection in rachitic infants has led to many studies in animals. Many of the earlier experiments in which cod-liver oil was used as the source of vitamin D are open to the criticism that this substance also contains vitamin A. The problem has been greatly clarified by the discovery of the specific anti-rachitic effects of ultraviolet light, and of irradiated ergosterol. Eichholz and Kreitmair (1928) fed five groups of thirty adult mice on 1, normal diet; 2, rachitogenic diet; 3, rachitogenic diets with 0.001 mgm. irradiated ergosterol daily; 4, normal diets plus beer-yeast, and 5, normal diets plus beer-yeast and irriadiated ergosterol. All the animals received intraperitoneal injections of pneumococci on the thirty-third day after the beginning of feeding the special diet. The death rate during the following week was about 50 per cent in groups 1, 3, and 4; about 70 per cent in group 2 and about 30 per cent in group 5. This experiment is mentioned because it is typical of much of the work in this field, and is open to the following criticisms. 1. Half of the "rachitic" animals had already died when the infection was induced; presumably many of the survivors were moribund. 2 The lack of uninfected controls. 3. The authors admit that many trials had to be made before the "correct infecting dose of pneumococci could be found;" infection caused by intraperitoneal injection of the pneumococcus is exceedingly fatal to mice.

In the study of the resistance of rachitic rats to infection, Robertson (1929) selected a strain of colon bacillus isolated from the nasal cavities of rats. The virulence and suitable quantity of the culture of this organism were determined in preliminary experiments. Infection by intraperitoneal route was selected, because subcutaneous injections were found not to be fatal. Of 263 rachitic rats, exposed to sunlight out of doors, 57 per cent survived infection; of 241 rats kept indoors, 32 per cent survived. Exposure to quartz lamp, although resulting in cure of rickets, was not as effective in raising resistance as was exposure to sunlight. Exposure to cold air alone was quite ineffective. Cure of rickets by irradiated ergosterol was somewhat effective, but surprising as it may seem, cod liver oil was not. It is, therefore, difficult to be certain that the effects observed, were due to the anti-rachitic action of the

various agents used. Moreover, it seems likely that much of the mortality can be ascribed to the fact that the animals were in a generally weakened state. In a brief paper, Robertson and Ross (1930) state that of 89 rats fed a rachitogenic diet including non-irradiated "muffets," 5 per cent survive infection by mouth with B. enteritidis; whereas, 30 per cent of 94 rats fed the same diet including irradiated "muffets," survived. The difference, 25 ±5.3 per cent, is significant. These authors (Ross and Robertson, 1931) also state that of 41 rats fed a rachitogenic diet and exposed to sunlight, 61 per cent survive oral infection with B. enteritidis; whereas 28 per cent of 32 rats survived when exposed through ordinary glass. The difference, 33 ±11 per cent, is barely significant. It is not clear from the context that the groups were strictly comparable. It will be seen that this is important, when one compares the mortality in the control groups in the two experiments—95 per cent in the first, 72 per cent in the second.

Freund (1931–32) has studied the effect of rickets upon infection in several species. Rats and mice on rachitogenic diets with large doses of irradiated ergosterol received intradermal injections of hemolytic streptococci; the resulting phlegmons and sepsis were no less severe in the protected animals than in the rachitic controls. Large doses of "radiostol" administered to a rabbit for some days before intracutaneous injection of streptococci appeared to limit the inflammatory reaction somewhat. Rachitic rats were infected with trypanosomes; immune human serum caused the disappearance of the organisms in 24 hours. In control animals, in which rickets had been prevented with radiostol, the parasites disappeared in 48 hours, but more of the rats suffered a fatal relapse. In this work, only a few animals were used.

Bradford and Boynton (1931) in comparing the resistance to infection of rats suffering from deficiencies of vitamin A and of vitamin D, used an organism isolated by Bradford (1928) from rats with vitamin A deficiency. Cultures were injected into the peritoneal cavity. These authors showed clearly that animals fed on diets low in vitamin A, had very low resistance, whereas those with rickets had as much resistance as normal rats. The loss of resistance in rats deprived of vitamin A appeared before any other evidence of vitamin A deficiency.

At the present time, therefore, it is not possible to make a general statement with regard to the lack of resistance to infection of rachitic animals. It seems likely that in certain species, rachitic animals are more susceptible than normal animals to certain types of infection: even this statement must be a guarded one; because the lack of resistance

may be due merely to the general weakness of the animal, and may have little to do with the lack of the specific vitamin D. On the other hand, rachitic rats have been shown to have quite normal resistance to certain infections.

Attempts to increase resistance in non-rachitic animals with vitamin D or ultraviolet irradiation have been stimulated by the recent discoveries of the specific activity of these agencies. Rossi (1931) states that enteritis of young calves, which is usually fatal, can be cured with irradiated ergosterol. Donohue (1932) induced fowl cholera artificially, with Pasteurella avicida, in chicks receiving C. L. O. Irradiation, in addition, carried out with the mercury vapor quartz lamp, was of slight benefit to the birds which had received subcutaneous inoculation. Hill and Clark (1927) studied the effect of ultraviolet irradiation of normal rats upon the course of pneumococcus infections. Irradiation after infection was without effect. Irradiation before inoculation had, apparently, a slight beneficial effect. In 1931, Hardy, Chapman and Clark showed that intranasal infections of pneumococcus in rabbits were no milder in animals which had previously received irradiation from a mercury vapour quartz lamp, and were definitely more severe if the animals were exposed to direct sunlight or to sunlight passed through vita-glass. Infection by Bacillus lepisepticum was slightly less fatal in the irradiated group. Hill, Greenwood, and Topley (1930) studied the influence of preliminary ultraviolet irradiation upon the resistance of mice introduced into a community of mice suffering from an endemic infection of Pasteurella. The expectancy of life when animals were added was calculated from the observed data of the life-table. Controls and irradiated animals—about 1000 in all—showed exactly the same period of survival.

It seems obvious that there is no good reason to expect that the resistance of non-rachitic animals to infection can be increased by anti-rachitic agents; neither is there, from the experimental work quoted, any very striking evidence for the general belief that lack of vitamin D predisposes to infection.

*Vitamin A.* McCollum in 1917, first pointed out that rats suffering from deficiency of vitamin A develop severe spontaneous infections. Drummond (1919) confirmed these observations. Mellanby (1919) drew attention to the liability to pneumonia of pups fed little fat-soluble A. McKay (1921) noted that kittens fed milk in which the fat has been replaced by olive oil, become emaciated and anemic and develop heavy infections with Dipylidium caninum. Daniels, et al. (1923) emphasized

the great frequency of purulent infections of the respiratory tract, in rats with xerophthalmia. In this earlier work, the diets used had often been deficient in vitamin D as well as vitamin A. McCollum and his co-workers in 1922 clearly showed that the anti-xerophthalmic factor in cod liver oil could be destroyed by oxidation without destroying the anti-rachitic factor; and Bloch (1924) demonstrated that sunlight cured rickets in children without curing xerophthalmia and the associated susceptibility to infection. Green and Mellanby (1928) provided vitamin D in the form of irradiated ergosterol in diets deficient in vitamin A; in their extensive series of rats thus protected against rickets spontaneous infections were no less frequent, and could be prevented by vitamin A or its precursor, carotene (Green and Mellanby, 1930) a daily dose of 0.04 mgm. preventing the development of all foci of infection in the young rat; a dose of 0.02 mgm. being partially effective. These authors claim a specificity for the action of vitamin A, by calling it "the anti-infective vitamin." This claim is open to the objection that in certain species of animals, resistance may be reduced by a deficiency of vitamins other than vitamin A.

Animals suffering from deficiency of vitamin A are more susceptible than controls to a number of infections artificially produced. Zinnser (1931) finds that normal rats are difficult to infect with rickettsia, whereas those with xerophthalmia, upon intraperitoneal injections, develop an exudate exceedingly rich in these organisms. Intestinal worms develop more readily in chickens (Ackert, 1929), dogs and cats (Foster and Cort, 1932), when the host is deprived of vitamin A. More complete studies have been made with bacteria of various kinds. Certain bacteria, saprophytic in normal animals, increase in number and may become pathogenic in avitaminotic animals (Bradford, 1928; Turner, 1930).

Infections are readily induced in animals with deficiency of vitamin A by artificial inoculation. The mechanism of infection with paratyphoid bacilli in normal mice has been established by Ørskov and Moltke (1928). Fed by mouth, the bacilli taken promptly disappear from the alimentary tract, and are then found only in the lymph nodes of the mesentery, and not at first in the blood stream. Apparently they pass next by the thoracic duct into the blood, causing a transient bacteremia. The liver, spleen, and peripheral lymph nodes remove them completely from the circulation. In resistant animals, the bacteria are destroyed in these organs. In animals with poor resistance, or in case of heavy infection, a secondary, severe, and often fatal infection of the blood stream ensues. The intestine may again become infected by way of the bile.

Ørskov and Lassen (1930) demonstrate how effective a barrier is furnished by the intestinal tract, together with its lymphatic tissue: 200,000 to 500,000 virulent paratyphoid organisms must be administered by mouth to produce as serious an effect as 5 bacilli injected under the skin. Lassen (1930) shows that in vitamin A deficiency of rats the course of infection is exactly the same. However, the secondary bacteremia, resulting from inability of the liver and spleen to destroy the bacilli is more severe and much more frequently fatal. In Lassen's opinion, the direct cause of death is infection, and not vitamin deficiency in itself.

In 1931 Lassen compared the effects of deficiencies of vitamins A, B and D, in rats infected with paratyphoid. In case of vitamin B, only the animals which were nearly moribund died more quickly than the controls; in case of severe rickets, there was no difference. In case of marked undernutrition only slight increase of susceptibility occurred, and this was not improved by cod liver oil. After intravenous injection, bacteria disappear as rapidly from the blood of A deficient animals as in the case of controls; but secondary fatal bacteremia was invariable, whereas with the dosage used, no fatal bacteremia occurred in control rats. Two criticisms of this work may be of interest: 1. The deficiency of vitamin A was far advanced when the infection was produced. No experiments were done before the stage of xerophthalmia had developed. 2. The "control" animals received a "normal" diet and not the deficient basal diet corrected by the addition of the vitamin. These criticisms have been met in the work of Bradford and Boynton (1931). Their control animals received a diet in which vitamin A was furnished by cod liver oil; the experimental animals received the basal A free diet to which irradiated ergosterol had been added. The animals received intraperitoneal injections of a culture of an organism isolated by Bradford (1928) from spontaneous abscesses occurring in rats with xerophthalmia. This organism is pathogenic only for such rats. These investigators showed that even before any signs of vitamin A deficiency appear, rats on a diet free of vitamin A, are much more susceptible than are the controls. This work has been confirmed by McClung and Winters (1932) using Salmonella infection in rats. Rickets produced much less fall in resistance than did a deficiency of vitamin A.

These experiments, together with those of Lassen, demonstrate that this susceptibility does not depend wholly upon metaphasia of the epithelium in the respiratory tract, as claimed by Wohlbach and others, because the infecting organisms were introduced into the peritoneal cavity. These experiments raise a question as to the existence of mild

forms of vitamin A deficiency: a deficiency not perhaps leading to any of the characteristic pathological changes, but yet, sufficient to lower resistance to infection.

The studies of Webster and Pritchett (1924) indicate that such a mild deficiency may exist. Groups of female mice and their offspring were fed three diets: A. The standard diet of the Rockefeller institute—bread, milk, a mixture of grains and dog-biscuit. On this diet, growth and reproduction for generations appear normal. B. The McCollum normal diet for rats—whole wheat flour 67.5 per cent, casein 15 per cent, dried milk powder 10 per cent, NaCl 1 per cent, $CaCO_3$ 1.5 per cent, butter 5 per cent of the mixture. C. A diet of bread and milk. Infection was produced by feeding a definite number of bacilli of mouse typhoid bacilli to groups of young mice. After 40 days, the mortality in the various groups was A, 70 per cent; B, 10 per cent; C, 80 per cent. After a second mating, the mortality of the infected young was B, 23 per cent; C, 100 per cent. It was also shown that the McCollum diet conferred a greater degree of protection against poisoning with mercuric chloride and against botulinus toxin. In an elaborate series of tests Pritchett (1927) determined that the superiority of the McCollum diet was due to the butter fat, which comprises 5 per cent of the mixture. When cod liver oil replaced butter, the protective effect was somewhat less. This may have been due to an injurious action of such a large quantity of this substance. Jundell and Agduhr believe that cod liver oil even in moderate amounts is injurious. Recently, large doses of more or less pure vitamin A have been reported to be toxic, and even to cause infection (Collazo and Rodriguez, 1933a, b; Drigalski and Laubmann, 1933). Pritchett shows that the protective value of butter fat varies with season, as one might expect its content of vitamin A to vary. This very suggestive evidence would have been completely convincing if, after the vitamin A of the butter had been destroyed by McCollum's oxidative procedure its protective action had also disappeared.

The studies just described form part of an elaborate study of experimental epidemics in large communities of mice. Webster (1930) has shown that in such epidemics, the virulence of the infecting bacteria may remain constant; and that diet is one of the factors controlling the onset and disappearance of an epidemic. In these experiments, the superior protective value of the McCollum diet as compared with the Standard Institute diet was again demonstrated. Topley, Greenwood and Wilson (1931) have also made observations on experimental epidemics. Twenty-five mice, infected with B. paratyphosus were intro-

duced into 2 cages each containing 100 uninfected mice, which had been for 14 days on the diet being studied. The mortality of the latter animals was observed for 60 days. The normal diet was whole rolled oats; milk and water, equal parts for 5 days a week, water only for 2 days a week. The experimental diets were mixtures resembling McCollum's, in which butter, lard and cod liver oil concentrate (20 times the value of C. L. O.) were varied. To the surprise of the investigators, these diets offered no superiority over the standard diet. The survival times were:

Standard diet: 50.4 ± 0.74 days.
Diet with 5.6 per cent butter fat: 48.2 ± 1.30 days.
Diet with 5.6 per cent C. L. O. concentrate: 38.1 ± 1.38 days.
Diet with 43 per cent lard: 43.8 ± 1.32 days.

Intraperitoneal injections were then carried out. The mortality rate was slightly, though not significantly, higher in the animals fed on a normal diet. In a final experiment the authors added either mangolds, cabbage or carrots to the normal diet, and determined the survival time of groups of 200 mice. The average life of mice receiving normal diet was 39.6 ± 1.2 days; the group receiving additions of cabbage, mangolds, or carrots survived 38.3 ± 2.0; 34.1 ± 3.40, and 30.0 ± 2.75 days respectively. This experiment suggests a doubt as to whether the consumption of relatively large quantities of vitamin A is likely to be a successful measure in combating bacterial infection; since the animals receiving cod liver oil concentrate or large amounts of butter, developed oily coats suggestive of hypervitaminosis A, it would appear that large amounts of vitamin A may actually hamper resistance to infection. It will be noted that the mice used by Pritchett and Webster had been consuming the experimental diet for more than one generation, whereas those of Topley, Greenwood and Wilson were put on the diet only 14 days before the experiment began.

The discovery of a reliable physical or chemical method for estimating vitamin A in tissues will make it possible to know whether such observations as those just recorded are really concerned with vitamin A; and if they are, to reconcile them. Certain observations made by McCoy and McCoord (1933) using the author's modification of the Price-Carr test, indicate that a diet deficient in vitamin A given late in life, and deficient enough to exhaust the store of vitamin A in the liver, may not greatly lessen the resistance of rats to trichinosis; whereas a deficiency in early life invariably does so.

*Summary.* Deficiency of vitamin A leads to loss of resistance to in-

fections. In the rat and the mouse, this loss of resistance seems greater than in cases where the deficiency is that of vitamin D or B. Whether this lowered resistance, due to lack of vitamin A, occurs in the guinea pig is not known, but the reaction of scorbutic guinea pigs to infection could with profit be compared with that of guinea pigs deficient in vitamin A. There exists a certain body of evidence that a partial deficiency of vitamin A in early life may lower resistance. It seems possible that excessive amounts of vitamin A may be injurious. At the present time, it is hardly justifiable to term vitamin A the specific "anti-infective vitamin," since a deficiency of vitamins other than vitamin A has been shown to lower to some extent, the resistance of animals to infection, while factors such as temperature and starvation have been shown to play a part.

*Water and minerals.* Clinicians have long believed that retention of water and salts in the tissues might lower resistance to infection. Some believe that diets low in sodium chloride promote resistance. The lack of animal experiments designed to test this view is astonishing. Hotta (1928) studied mice receiving diets with varying amounts of sodium chloride and vitamins B, C, A (together with D). Cultures of mouse-typhoid were injected into the peritoneal sac. Diets low in salt and in vitamin A afforded the *least* protection.

Jackson and Smith (1931) restricted the water-intake of rats to such a degree that no increase in weight took place for several months. The controls were given water ad libitum, but only as much food as the experimental rats ate; the growth of the latter group was from 33 per cent to 98 per cent of normal. Both groups were emaciated, but no tendency to infection was noted in either group. Mikoshiba (1931) states that acidosis has no direct effect on bacterial infection, but may occur during an inadequate response to infection. Bonanno (1932) finds that guinea pigs receiving such acid-producing salts as calcium chloride or ammonium chloride, begin to die 15 days after intraperitoneal injections of tubercle bacilli; those receiving alkalis, such as sodium bicarbonate or sodium citrate begin to die 20 days after the injection; whereas the controls survive for the first two months.

*Combinations of dietary factors.* Several reports have been published on the anti-infective value of complex food products prepared from animal tissues. As the composition of these products is not definitely specified, and as they are apparently trade secrets, they cannot be considered here.

Chen and Li (1930) have compared the anti-infective values of vege-

tarian and normal diets for the rat. The vegetarian diet consisted of whole wheat 35, millet 30, soy bean 15, peas 15, bean-oil 5 parts; sodium chloride, 1 gram per 100 grams; fresh cabbage ad libitum. The normal diet consisted of whole wheat 66, whole milk powder 33, sodium chloride 1 part. Hog-cholera bacilli were injected into the peritoneal cavity. The mortality within 2 weeks was 42 ± 8.9 per cent in the normal group; and 84 ± 6.6 per cent in the vegetarian group. The difference, 42 ± 11 per cent is significant. In other experiments, using an intraperitoneal injection of pneumococci, these authors found a lower mortality in the group of animals fed the vegetarian diet, though in this experiment the difference in the mortality between the normal and vegetarian groups is not significant. Whatever may be the reason for these differences, the experiments illustrate the importance of the nature of the infecting organism.

Pfannenstiel and Scharlau (1930a, b) reported that abscesses caused by injecting hemolytic staphylococci into the skin of rabbits, may be greatly limited in extent and rapidly cured by treatment with a combination of vitamin D, calcium gluconate, and dried yeast. The dose of irradiated ergosterol was large enough to produce mild toxic symptoms. The quantity of yeast, 8 grams per day, was also large; it would be equivalent on the basis of body weight to about ¼ lb. for an adult human being. In his next publication, Pfannenstiel (1931a) used a proprietary mixture of the materials above mentioned. The number of animals used was small. Schubert (1931a, b) using a somewhat larger group of rabbits, was quite unable to obtain any benefit; even when at the suggestion of Pfannenstiel (1931a, b) he used very large doses of irradiated ergosterol. It is obvious that the remedies mentioned, when given in the doses advocated, are not foods, but drugs; and might, if given indiscriminately to man, lead to serious symptoms.

Allen (1932) has investigated in newly-hatched chickens, the effect of diet upon the course of infection with Eimeria tenella. Among those receiving a diet high in protein and in vitamins A and B, fewer oöcytes appeared in the stools at first, there was less hemorrhage, the bleeding was of shorter duration, and the mortality was much lower.

It is apparent that where so many dietary factors vary, results must be difficult to analyze, almost impossible to interpret, and of most uncertain trend.

*Diets low in caloric value or in proteins.* Trommsdorf (1906) reviewed the literature upon the effect on resistance to infection of changes in external temperature, of starvation, of thirst, of bleeding, of toxic sub-

stances, and of the removal of certain organs. He contributed his own studies of the effect of cold, starvation, fatigue, and alcoholic intoxication. Only when these influences were very marked could a depressing effect be observed, and not always then.

Jägerroos (1902) kept two dogs, for a very long period, on a diet very low in protein. Both dogs, becoming infected, appeared to show very little resistance.

Thomas (1919) attempted to show that suckling pigs resist an infection better if the diet is rich in protein from the beginning. They do not resist infection better if such diet is given late in the infection.

HUMORAL RESISTANCE TO INFECTION AND DIET: a. *Transmission of antibodies in colostrum and milk.* The very extensive literature on this subject has been summarized by Bauer (1910) and by Kuttner and Ratner (1923). It is generally agreed that milk never contains more than a trace of immune substances. In man and in rodents, immune bodies pass readily through the placenta and are not readily secreted into the colostrum. Kuttner and Ratner could find no diphtheria antitoxin in human colostrum. Debré and co-workers (1930) immunized 6 women during pregnancy with tetanus toxoid. The concentration of antitoxin in their blood rose; but appreciable amounts were detected in the colostrum only in one case.

In goats and cows the placenta is said not to transmit antibodies, whereas large quantities are found in the colostrum. Theobald Smith and his co-workers (1922a, b; 1923; 1924a, b; 1930) have demonstrated that the colostrum of the cow contains antibodies for the colon bacillus. These antibodies are absorbed from the intestine of the calf; and protect it against colon-bacillus sepsis, which is usually fatal. The serum of the newly born calf contains neither euglobulin nor pseudoglobulin I, proteins which it obtains from the colostrum, and with which the antibodies are associated.

b. *Influence of diet on antibodies of serum.* In attempting to explain the changes in resistance to infection brought about by changes in diet, many investigators have studied the various antibody reactions of the serum. The results are by no means concordant. The early work is reviewed by Müller (1903, 1904); who studied the formation of agglutinins by injecting various kinds of killed bacteria into pigeons, which were markedly underfed. The titre of agglutinins became higher in starving birds than in the controls to B. typhosus, B. pyocyaneus, and B. dysenteriae; and lower to the vibrio of Metchnikoff and to B. proteus. As the author points out, this does not mean that starved pigeons

are more resistant to inoculation with B. typhosus, for, as he showed in this study, the contrary is true.

The hemolytic complement of the serum in rabbits nursed by the mother is higher than in those artificially fed (Heimann, 1908).

Precipitins for foreign protein are not readily formed by very young animals; but they acquire the ability to produce them at an earlier age, if fed artificially (Moll, 1908; Ossinin, 1913). No attempt was made to explain this.

Kleinschmidt (1914) studied the production of hemolysins for red corpuscles of sheep, typhoid agglutinins, and typhoid bactericidins in dogs. Adult dogs proved too variable. Pups of the same litter were finally used. Diets excessive in carbohydrate or fat, feeding with cow's milk or by the mother, led to no striking differences in antibody formation. Usually, hemolysin formation was lower in pups fed artificially, but it was sometimes normal even in severe nutritional disturbances. Hektoen (1914) fed rats on a diet, the protein of which was furnished only by corn-gluten. Although their growth was retarded, they were capable of forming hemolysin for sheep corpuscles as well as were the controls. Zilva (1919) studied the production of agglutinins, complement, and amboceptor in rats. The separate dietary deficiencies included those of the minerals Fe, Ca, K, Na, Cl and P; of protein; and of vitamins A plus D, B, and C. Only in the animals whose diets were deficient in phosphorus could he consistently observe poor production of agglutinins for B typhosus and amboceptors. Wolff and Lewis (1919) report that Fleischman's yeast added to the normal diet of adult rabbits exerts no influence upon their ability to produce antibodies. According to Hamburger and Goldschmidt (1913) scurvy in guinea pigs and in human infants does not lessen bactericidal power, ambocepter or complement. In dehydration complement rises only when the serum protein rises. Smith and Wason (1923) found normal values for complement in rachitic rats; but low values for bactericidal activity towards B. typhosus. Perhaps the publication most frequently quoted is that of Werkman (1923a, b, c) who gives a critical review of earlier papers. In the rat, rabbit, and pigeon he was unable to find any significant differences in formation of antibodies as a result of diets deficient in vitamins A or B. Agglutinins, precipitins, hemolysins, and bacteriolysins were studied. The opsonic index studied in vitro was not altered, but phagocytosis of bacteria injected into the peritoneal cavity was definitely low in animals deficient in vitamin B or in vitamin A. The difference between the index in vitamin B deficiency and in controls was $1.1 \pm 0.23$,

in case of vitamin A deficiency the difference was 1.1 ± 0.24. This together with the greater susceptibility to injections of bacteria, was ascribed by the author to lowering of the body-temperature.

Simola and Brunius (1933) devised a basic diet devoid of vitamins A, C, and D, suitable for the guinea pig. These vitamins can be supplied by adding carotene; lemon juice; or vigantol. When the animals had developed signs of the desired deficiency, the serum-complement was determined, as well as the ability to form hemolysins for sheep-corpuscles. In none of the deficiencies, nor in hypervitaminosis D, was any difference observed.

Findlay and MacLean (1925) criticize the work of Smith and Wason (1923) because rats normally have very little ability to form antibodies against B. typhosus. Using a diet in which the ratio of phosphorus to calcium was such as to prevent rickets in the rat and which was devoid of vitamins A and D, Findlay and MacLean found that the serum maintained its normal power to kill staphylococcus aureus, until after the animal had developed xerophthalmia. The serum of rachitic rats killed 45 per cent of added S. aureus; that of control rats, 71 per cent. (See earlier work of Zilva.)

Bieling (1926) distinguishes antibacterial immunity from antitoxic immunity. The former was not affected in underfed rabbits, whereas antitoxin immunity was diminished. As he states, it is well recognized in the manufacture of antitoxic serums that the animals should be well nourished.

Orr, Macleod and Mackie (1931) have studied in the serum of the sheep, normal lysins of B. coli and B. suipestifer, and agglutinins of B. abortus, and B. paratyphosus; all of which were diminished when the animals were placed on poor pasturage. Eighty castrated males 7 months old were fed a diet poor in protein and calcium; half of these animals were kept indoors about 4 months, and there ceased to grow. Calcium salts, and especially cod liver oil, brought about growth. Seasonal variations in the titre of the serum occurred. Lysins for B. coli, and agglutinins for B. abortus were highest in March and lowest in September. Agglutinins for B. paratyphosus were highest in January and September and lowest in May. Calcium salts and cod liver oil and especially access to grass increased the titre of the lysins for B. coli. The animals in the worst condition, and those on diets lowest in protein, possessed the highest titre of agglutins for B. abortus.

The rabbit was selected by Green (1933) for study because it readily forms antibodies, and because blood from it can be easily obtained

repeatedly. Normal hemolysin for sheep-corpuscles are not changed in xerophthalmia or rickets. Hemolysin for ox-corpuscles is very poorly formed by rabbits suffering from avitaminosis A, but is formed in a normal amount by the rachitic rabbit. In avitaminosis A, typhoid agglutinins are formed almost to a normal degree; hemagglutinins, and serum-complement are not affected. The formation of none of these antibodies is affected in rickets.

*Summary.* The majority of writers agree that both the normal antibodies of the serum and the power to form antibodies are not affected by dietary deficiencies; a few exceptions have been reported. More study should be directed to the effect of deficiency of minerals upon the humoral antibodies.

c. *Effect of nutrition on antibodies in man.* Some years ago, Moro (1907) studied the hemolytic complement of the serum of infants, showing that it was diminished in acute infection, and claiming that it was higher in breast-fed infants. Kaumheimer (1909) found that those cases of Moro which had had highest titres of complement had subsequently thrived the best. Complement increases with age, as does amboceptor (Gerwinn, 1908–1909): Amboceptor makes its appearance earlier in the bottle-fed baby (Bauer and Neumark, 1910). Normal bactericidal action of the serum can be demonstrated even in premature infants; and in them, especially, is liable to be much diminished by nutritional disturbances (Kyrklund, 1921). Osborne (1933) found that cod liver oil in a large single dose (50 cc.) increases the hemolytic complement 5 hours afterwards in the serum of adults.

d. *Lysozyme.* A substance capable of dissolving certain bacteria of the air was discovered by Fleming (1922) in tears, fresh egg-albumin, and many tissues. This substance has a number of properties which distinguish it from ordinary bacteriolysins, notably its greater resistance to heat. In animals suffering from xerophthalmia, it disappears from the conjunctival sac; the application of it to the eye delays and may prevent the development of xerophthalmia in rats receiving a diet deficient in vitamin A. Very possibly this substance is identical with that described by Weil (1911). It is present in human colostrum and milk, but not in cow's milk; it is thought by Rosenthal and Lieberman (1931) to be of importance in the development of the intestinal flora of the newly born infant. Anderson (1932) has shown that lysozyme was much diminished in the tears of two children with xerophthalmia; and during appropriate treatment with cod liver oil, that it rose remarkably within 5 to 7 days.

RESISTANCE OF TISSUES. The loss of resistance to infection observed in several dietary deficiencies cannot be ascribed to a loss of circulating antibodies, or to a failure in their production. For these phenomena often do not occur, even when the animal has become lacking in resistance. It would seem more fruitful to study the resistance of the tissues themselves. It is known that bactericidal substances are liberated from injured tissues (McCowen, 1926) and consequently exist there in high concentration. Saxl and Donath (1927) describe a technic which might with profit be applied to the study of the bactericidal activity of tissues in dietary deficiency. Some years ago Ronzani (1908) studied the bactericidal activity of the lungs of guinea pigs, which had inhaled droplets containing B. prodigiosus. Counts were made of the bacteria surviving in weighed portions of the lung, at different intervals. He found no change due to overheating, but a fall as the result of exposure to cold, fatigue, trauma, and inhalation of dust. The effect of diet was not studied.

Animals receiving deficient diets may become more susceptible to the injection of bacterial toxins. Werkman and his co-workers (1924) showed that rats suffering from deficiency of vitamins A or B are more susceptible than control animals to diphtheria toxin, although the ability of such rats to produce antitoxin is not diminished; also the rate at which injected toxin disappears from their blood is normal. Rats on normal diets resist the action of tetanus toxin, but when fed "deficient diets" according to Bieling (1925) do not; Blackberg (1928) reports that complete deficiency of vitamins A and D, and of vitamin B in the rat, reduces the resistance to tetanus toxin to 1/40 or 1/100 of the normal. The reaction of the skin of sheep to injection of the toxin of the bacillus of lamb dysentery is more violent in animals which have received a diet low in minerals and vitamins A and D (Mackie, et al., 1932). In attempting to account for seasonal variations in the response of guinea pigs to diphtheria toxin Torrance (1933) finds a correlation of $0.456 \pm 0.105$ between the survival time and the vitamin A content of the liver.

The loci where bacteria may be supposed to be destroyed in the organism are: 1, the skin; 2, the mucous membranes of the respiratory tract and of the gastro-intestinal tract; 3, the blood; 4, the lymph nodes; 5, the serous cavities; 6, the liver and spleen. Few studies have been made of the resistance of the peritoneum and lymph nodes in animals with dietary deficiencies; none have been made of the resistance of the pleura, or skin.

The leukocytes may be affected in dietary deficiency. According to

Lawryncowicz (1931) scurvy in the guinea pig is associated with a fall in the granulocytes; and the phagocytic power is diminished. Intraperitoneal injections of irritating substances failed to produce an exudate clouded by pus-cells. Turner and Loew (1931) show that rats suffering from deficiency of vitamin A have normal numbers of white blood cells and a normal differential count, until a terminal leukocytosis appears. Hennessey (1932) studying the inmates of jails in Uganda, observed vitamin A deficiency which caused no change in the blood picture.

Lymphatic tissue is said to atrophy in animals deprived of vitamin B (Cramer, Drew and Mottram, 1921; Cramer, 1922); but no adequate correlation has been made between such atrophy and loss of resistance, if any, in such animals. Findlay (1923) showed that scorbutic guinea pigs are much less resistant to inoculation with pneumococcus, B. coli, Staphylococcus aureus, and Streptococcus hemolyticus. He observed a diminished cellular response in the blood and in the bone-marrow in such animals.

The duodenum and upper portions of the ileum are nearly free of bacteria in health. In starvation and in diarrhea these regions may contain bacteria (More, 1906). Seidmon and Arnold (1932a, b) have found that the entire intestinal tract contains an increased number of bacteria in rats deprived of vitamins A or B. Bacillus typhi murii, fed to rats, appears in greater numbers in the organs of rats deprived of vitamin A than in the organs of control animals.

The characteristic lesion in animals suffering from a deficiency of vitamin A is the replacement of the specialized epithelium of the respiratory, the gastro-intestinal, and urinary tract by squamous cell epithelium; and atrophy of certain glands (Wolbach and Howe, 1925). Cyst-like formations result at the base of the tongue, and obstruction occurs in the urinary tract due to accumulation of cellular material. It is obvious that such lesions may readily give rise to infection (Arons and van der Rijst, 1932); and that in some cases the cysts may give the erroneous impression of being abscesses. Harris, Innes, and Griffith (1932) found that spontaneous infections in rats receiving diets devoid of vitamin A do *not* occur unless keratinization of the epithelium has taken place. In some animals, however, spontaneous infection following deficiency of vitamin A is not the rule (monkeys, for example). It is, however, possible that keratinization may be hastened by infection; in this case, it would be necessary to assume that some loss of resistance had already occurred. Injury of the nasal mucous membrane of rabbits by phenol (Fisher, 1929) renders them highly susceptible to inoculation with bac-

teria from human infections. Silver nitrate and mustard-oil were used by Wámoscher (l. c.) to incite spontaneous nasal infections in rats. The possible local formation of antibodies in the mucous membrane by the method of Walsh, Sullivan and Cannon (1932) might with profit be studied in avitaminotic rats.

Although the weakened resistance to infection of abnormal mucous membranes following a deficiency of vitamin A is well established, it has been shown that such animals also have diminished resistance which can not be explained on the basis of injured epithelium, only. This has been demonstrated by intravenous and intraperitoneal injections of bacterial cultures. The experiments of Bradford and Boynton, of McCoy, of Ørskov, of Lassen and of others have already been described. It seems likely that one important factor in the failure of resistance in these experiments is the inability of the tissue-phagocytes, particularly in the liver and spleen, to retain and to destroy bacteria. The phagocytosis of bacteria by these tissues is probably normal (Lassen, 1931). In the literature dealing with the reticuloendothelial system, little is found concerning the effects of deficient diet. Aschoff (1924) mentions only a "possible injury in scurvy." Jorstad and Johnson (1926) state that lipoid solvents injected into normal animals call forth a very active local phagocytic response, which does not occur in animals fed on diets low in vitamin A. Injury of the reticuloendothelial system by colloidal copper (Króo and v. Janskó, 1931) profoundly limits the rate at which bacteria leave the blood stream, and permits a more early and more rapid secondary bacteremia (Oelrichs, 1932–33). It would seem therefore profitable to study more carefully tissue reactions to bacteria in animals suffering from dietary deficiency, and in such studies to include not only epithelial tissues, but those of the reticuloendothelial system.

Tuberculosis in experimental animals. 1. *General comment.* Chronic tuberculosis, as seen in man, has rarely, if ever been produced in experimental animals. The guinea pig is so highly susceptible to human strains of the tubercle bacillus, that the disease in this animal can always be regarded as acute. Partial protection with B. C. G. can, however, be accomplished in the guinea pig, and produces a more chronic disease; the study of the influence of defective diets in such animals has not yet been reported. Many experiments have been made with the acute disease; and some with infections with "attenuated strains" of tubercle bacilli. The rat, on the other hand, is so resistant to inoculation with tubercle bacilli, and the lesions produced with enormous doses of bacilli are so unlike those in man, that the application of experimental results to

man, based upon the experiments with the rat, must be accepted only with the greatest reserve (see Gloyne and Page, 1923). In another respect, also, the experimental work appears inadequate. Most attention has been focused upon diets completely defective in some particular; diets which lead more or less rapidly to the death or general weakness of the animal; whereas the tuberculous infection, of itself, is much slower in its course. Relatively little attention has been given to partial deficiencies of diet, combined with a tubercle infection likely to run a chronic course.

2. *Protein and minerals.* Mouriquand, Michel and Bertage (1923) state that a diet defective in fats, vitamins, salts, and proteins did not favor the more rapid development of tuberculosis in guinea pigs. In rats, heavily inoculated with bovine tubercle bacilli, Lange (1925) finds no influence of diets high, adequate, or low in protein. Diets low in NaCl and $CaCO_3$ favored the development of a more diffuse and more intense local reaction at the site of inoculation, a slower dissemination and a slower elimination of the bacilli. This latter result, however, could not be repeated (Lange, 1927). No other reports have been found with regard to the effect of NaCl. This is of some importance, because of the claim of Gerson that salt is injurious in human tuberculosis.

3. *Vitamin C.* Mouriquand et al. (1923), believed that scurvy and tuberculosis in the guinea pig were without influence one upon the other. Leichtentritt (1924b) discussed the earlier reports, and found that lemon juice prolonged considerably the lives of tuberculous guinea pigs fed on a scurvy producing diet.

Bieling (1924a) produced chronic tuberculosis by inoculating guinea pigs with tubercle bacilli attenuated by treatment with quinine derivatives. Such animals lived for two years, and had well localized lesions in the liver and spleen. Scurvy-producing diets led to the death of such animals in five to eleven days, whereas non-tuberculous controls lived three or four weeks. This experiment was designed to account for the high death-rate of tuberculous persons during periods of bad nutrition in Germany during the war. In this report, Bieling makes no comment on the presence of scorbutic lesions in his animals, nor upon the probable cause of their death. Careful histological examination in a series of animals with more acute tuberculosis (Bieling, 1924b) revealed scurvy in one instance, ten days after the diet was started, but no scurvy in six others. Bieling, however, believes that such animals are to be compared to human cases of "latent" scurvy. He suggests as an explana-

tion, a state of "anergy," basing his view upon the known decrease of the dermal reaction to tuberculin in scurvy as observed by Prausnitz and Schilf (1924). (See also: Schütze and Zilva, 1927.) Although tuberculous scorbutic guinea pigs may have a diminished dermal reaction to tuberculin, they are killed by the injection of doses only $\frac{1}{2}$ to $\frac{1}{3}$ of those fatal to non-scorbutic tuberculous guinea pigs (Bieling, 1925). From further reports of Bieling (1924c, d) it appears that the "normal" and "scurvy-producing" diets were different in so many particulars, that the results are not necessarily to be attributed to the absence of vitamin C. He did not, for example, use a diet for the controls consisting of the scurvy-producing diet *plus* orange-juice. This question bears directly upon the clinical practice of the use of antiscorbutics in the treatment of certain types of human tuberculosis. It could be settled by the analysis of organs by chemical methods (adrenals, especially), for vitamin C; and by the use of pure ascorbic acid.

Heymann (1925) induced subacute scurvy in tuberculous guinea pigs. These animals lived 73 days, the tuberculous controls living 141 days. Death was due to scurvy, rather than to tuberculosis.

The experiments so far discussed deal with animals already tuberculous, in which scurvy is induced some time later. Sewal et al. (1927) have attempted to ascertain whether a diet for guinea pigs, relatively low in green cabbage, will render the animals more ready to contract tuberculosis by contact with infected animals. Males only were used. Although the 97 animals on the deficient diet contracted pneumonia, they did not appear to contract tuberculosis. No controls on a normal diet were studied. McConkey and Smith (1933) have shown that in the majority of cases, guinea pigs, maintained on a diet low in vitamin C, develop ulcerative lesions of the intestine if fed daily doses of tuberculous sputum. If this deficient diet is supplemented by adequate amounts of tomato juice, the animals almost invariably remain free from intestinal tuberculosis. They stated that cod liver oil was not protective. It must be pointed out that guinea pigs require very little vitamin A and that small amounts were undoubtedly present in the basic diet.

Hagedorn (1928) has produced a type of tuberculous disease, unlike that of man, by intraperitoneal injection of 20 mgm. of bovine tubercle bacilli in rats. A basic diet was devised, free of vitamins A and D, B and C. He found that absence of the A and D component (cod liver oil) decreased the time of survival more than did either of the other deficiencies. Vitamin B complex (brewer's yeast) had little effect. Vitamin C (orange juice) had a definite protective effect. It is, however, not

generally believed that rats require vitamin C; and the question is raised by Hagedorn's experiments of the presence in orange juice of some protective activity other than vitamin C. Here again, the actual determination of ascorbic acid in the tissues or its use in feeding is indicated.

4. *Vitamin A.* The value of fat and fat-soluble factors in preventing and treating tuberculosis is suggested by clinical experience. Weigert (1907) studied suckling pigs, because they were easy to feed on diets which varied greatly in composition. He found much greater resistance to experimental tuberculosis in animals fed on cream mixtures; and comparatively low resistance in those fed on skim milk.

As already mentioned, the guinea pig, which has often been selected for study because of its great susceptibility to tuberculosis, is not altogether a satisfactory animal for experiments with vitamin A, to the absence of which it seems comparatively resistant. Leichtentritt (1924a) found that cod liver oil has no protective value in tuberculosis of the guinea pig. After showing that large doses of orange juice (30 cc. per day) apparently enable the guinea pig to "learn to live with his tuberculous infection," Leichtentritt (1924b) called attention to the injurious action of cod liver oil in this animal. He mentioned the fact that Czerny (1912) had noticed changes in the skin of tuberculous infants receiving 80 grams of cod liver oil a day. M. I. Smith (1923) has been unable to find any marked effect of administering cod liver oil to tuberculous guinea pigs receiving normal diets, or those receiving diets deficient in fat-soluble vitamins. In the latter case, beading of the ribs occurred, a sign of rickets which could be prevented by cod liver oil, without influencing the progress of the tuberculous infection.

Although the young rat is very liable to develop classic symptoms of deficiency, when deprived of vitamin A, it is not an animal very susceptible to tuberculosis. Lange (1925) studied adult rats which had for four weeks received a "routine diet" and were then infected by intraperitoneal injection of bovine tubercle bacilli. The group of animals were then fed diets deficient in: 1, fat soluble vitamins; 2, water soluble vitamins; 3, large quantities of fat, devoid of vitamins; 4, large quantities of butter; 5, diets considered "adequate." No marked differences were observed in the course of the infection. Very probably, the adult rats had stored considerable quantities of vitamin A before the experiment began. Smith and Kendrick (1926) studied rats aged one month, reporting that death occurred in the group receiving a diet low in vitamin A much earlier ($\frac{1}{3}$ of the time) than in those fed on a normal diet.

Complete protocols are not presented. As already mentioned, Hagedorn (1929) had shown that the mortality in tuberculous rats is higher in those deprived of vitamin A than in those deprived of vitamin C or of the vitamin B complex.

M. I. Smith (1928) reports that the normal rat tolerates the injection of 40 cc. tuberculin per kilogram of body weight; the tuberculous rat receiving normal diet tolerates 10 cc.; the tuberculous rat deprived of vitamin A, tolerates only 1 cc. Schütze and Zilva (1930) agree in this, but point out that such rats are extremely sensitive to other toxic substances, such as killed suspensions of Salmonella.

Finkelstein (1932) has studied mice, in which tuberculosis is said to produce the same lesions as in man, and to pursue a chronic course. His protocols indicate clearly that deficiency of vitamin A leads to a much higher and much earlier mortality from tuberculous infection than that which occurred in controls. The evidence he produced clearly indicates that death in the infected animals was not due merely to a greatly weakened state in the animals with defective diet. As source of vitamin A, Finkelstein used pure carotene.

5. *Vitamin D.* The discovery of the anti-rachitic effect of irradiated ergosterol has made possible the use of vitamin D free of vitamin A. Many attempts are being made to cause healing of tuberculosis and calcification of caseous lesions with this new preparation. Much of this work has been concerned with very large doses administered to animals receiving normal diets. Spies (1930, 1931) has shown that doses of viosterol large enough to cause abnormal deposition of calcium in the organs of the rabbit will also cause calcification in necrotic centers of tubercles, caused in these animals by bovine bacilli. The administration of repeated large doses of viosterol to guinea pigs with acute avian tuberculosis caused little increase in the calcification of the lesions, whereas considerably more occurred in the lesions of acute bovine tuberculosis (Spies and Walker, 1931; Walker and Spies, 1931). Jampolis and Witt (1933) criticized the use of the rabbit, on the ground that it is abnormally sensitive to irradiated ergosterol. Using the guinea pig, infected with virulent tubercle bacilli, they were able to obtain calcification of the lesions but not of normal tissue, by using carefully standardized doses of viosterol. The dose, however, if applied clinically would have been equivalent to 300 cc. viosterol 250 D, in a child weighing 22 pounds. De Savitsch et al. (1933) believed that healing is promoted more effectively by combined administration of viosterol and tuberculin. Ultraviolet irradiation is not curative in acute tuberculosis in the guinea pig (Luce-Clausen and Bayne-Jones, 1931).

Few studies are available on the effect of rickets upon tuberculosis, possibly because experimental rickets is not so well known in the guinea pig as in the rat. Grant et al. (1927) claimed that rachitic rats may be more readily infected with tubercle bacilli than normal controls.

6. *Combination of dietetic factors.* Agnes Grant (1930a, b) claimed that in rabbits neither vitamin D nor vitamin B alone confers protection, whereas their simultaneous administration does. There was distinct evidence in these animals of hypervitaminosis D. The difficulty in working with a large number of variable factors would necessitate the use of many more animals than have been used in any of these experiments.

*Summary.* Beyond question, scurvy lowers the resistance of the guinea pig to tuberculosis; and deficiency of vitamin A may lower the resistance of the rat and mouse to tuberculosis. It has not yet been shown that rickets lowers the resistance of animals to tuberculosis, nor that intensive treatment with vitamin D is beneficial. More rapid progress will be made when the human type of tuberculosis can be produced in animals.

TUBERCULOSIS IN MAN. In view of the great difficulty in imitating the human type of tuberculosis in animals, and of applying to man the results of animal experiments in disorders due to dietary deficiency, the dietetic treatment of human tuberculosis must rest upon clinical experience. Interpretation of clinical observation is perhaps more difficult in tuberculosis than in any other chronic disease.

1. *Dietary factors predisposing to tuberculosis.* As a result of long observation in his private practice, Vaile (1927) stated his belief that persons who habitually consumed diets high in fat are less liable to develop tuberculosis. As such diets are invariably more costly than diets high in carbohydrate and low in protein, it is more than likely that social and economic distinctions must have existed between the two groups. Gordon and Tai (1931) note that for a period of one or two years before the onset of tuberculosis, the majority of their patients had been accustomed to eating diets extraordinarily low in energy value—averaging 1400 calories per day, and sometimes as low as 800 calories. These diets were especially lacking in dairy products and green vegetables. Among their patients, the children, however, had had diets more nearly adequate in total energy value, but lacking in "accessories." In all patients, respiratory infections were frequent.

2. *Vitamin A.* The observations just cited, together with the work of Weigert (1907) who showed that experimental tuberculosis pursues

a milder course in suckling pigs fed cream, than in controls fed skim milk, at once suggests that a relative deficiency of vitamin A may be an important factor affecting the course of human tuberculosis. Wagner and Happ (1923) report that 9 children with tuberculosis made better progress during the periods in which they received diets rich in fat (butter and cream) than in periods in which they received an isocaloric diet poor in fat. Cod liver oil was not used. During the war, Denmark exported her dairy products, and xerophthalmia became endemic. The death-rate from tuberculosis at that time reached the highest level observed for many years, and fell promptly when laws were passed limiting the exportation of dairy products. Pattison (1930) administered a concentrated preparation of vitamins A and D to 43 patients with bone tuberculosis. This concentrate contained about 20 times as much vitamin A as does cod liver oil. To 35 patients, cod liver oil was given. No differences were noted in the clinical improvement (74.4 per cent in the first group, 71.4 per cent in the second group, improved). As cod liver oil had been given before the experiment began, both groups had already had an optimal amount of vitamins. Gordon and Titherington (1923) injected subcutaneously, in ambulatory tuberculous patients, a concentrate of vitamin A (1 cc. containing 4000 U. S. P. units) three times a week for 19 weeks. Although some of them improved, the authors do not feel that the effect was in anyway "specific" in promoting calcification of tuberculosis.

We may therefore conclude that diets deficient in vitamin A may predispose to tuberculosis in man, or aggravate its course; and that an optimal intake of vitamin A exists, and is provided by the diets now in use; but that there is no evidence at present that increasing the intake of vitamin A beyond the optimal amount is in any way necessary.

3. *Vitamin D.* To 9 children consuming normal diets, and suffering from bone tuberculosis, Kramer, Grayzel and Shear (1929) administered 4 mgm. of irradiated ergosterol daily for four months, and 8 mgm. daily for the next eight months. Nine control patients received no irradiated ergosterol. Healing was not promoted, there were no harmful effects, and the Ca and the P of the serum were not changed. Similar negative results are reported by Kaminsky and Davidson (1931) in pulmonary tuberculosis. In the cases of Gordon and Tai (1931), already mentioned, the administration of brewer's yeast and of a concentrate of vitamins A and D appeared to improve the resistance to respiratory infections, in those who gained weight, but had no influence on the calcification of tubercles. Scheurlen and Orlowitsch (1930, 1931) be-

lieve that the modern diet contains an optimal amount of vitamins D and B; and find no improvement whatsoever which can be attributed to giving "vigantol" and yeast.

It is beyond the province of this review to discuss the effect of ultraviolet irradiation in tuberculosis. It is generally believed that tuberculosis of the peritoneum and of the bone is benefited; and that pulmonary tuberculosis often is not. It is not known whether the effect in tuberculosis is due to the anti-rachitic action of ultraviolet irradiation.

4. *Vitamin C.* McConkey (1930) reported that tuberculosis of the intestine is not benefitted by cod liver oil, but is improved by ultraviolet irradiation; a much better result is obtained by the use of cod liver oil and orange juice or tomato juice. This effect may in part be due to the vitamin C. The inferences of Stub-Christensen (1929–31) would seem to indicate the great importance of vitamin C, but no definite proof is given. He observed a rapid decline in the morbidity rate of tuberculosis in Denmark in April, when new potatoes are suddenly introduced into the diet. But as he points out, other dietary factors, especially vitamin A, are likely to be introduced at the same time. It would also seem that respiratory infections which aggravate tuberculosis must be decreasing at this season. So far as the reviewer is aware, no definite study has been made of the use of diets high in vitamin C, but not in other vitamins, for the treatment of tuberculosis. This is remarkable, in view of the evidence supplied by the experimental work with scurvy and tuberculosis in animals.

5. *Gerson-Sauerbruch-Hermannsdorfer diet (G-S-H).* This diet, elaborated by Gerson of Bielfeld, was introduced to the medical profession by Sauerbruch (1926), professor of surgery at the University of Munich. Sauerbruch's assistant, Hermannsdorfer, had already developed independently many of the features of the diet to promote the healing of surgical wounds. At Sauerbruch's invitation, Gerson introduced the diet into the Munich clinic where it was most vigorously championed. The chief articles of food in this diet are: much cream and butter; vegetables; fruits; eggs; cod liver oil with phosphorus; little meat; very little sodium chloride (about 1.6 gm. per day). The energy value is about 2800 calories; the food constituents include approximately—protein 67 grams, fat 170 grams, carbohydrate 235 grams. The ash is alkaline. Great stress is laid upon "transmineralization," a special (proprietary) mixture is given each day, rich in alkaline earths and phosphorus, and devoid of chlorides. In Germany, the reception of this diet was enthusiastic (Schüller, 1928; Sauerbruch and Hermannsdorfer, 1928;

Alexander, 1930; Bommer, 1932; Sellei, 1932) and to some extent in other countries (Mayer and Kugelmass, 1929). These reports, however, make no mention whatever of any attempts to establish by experiment in man the value of any of the various constituents. Most of the reports deal with series of cases, which the authors considered to be severe; no control cases treated in any other manner are compared with the cases treated by the G. S. H. diet. Boldt (1932) finds no advantage in this diet for children. Stub-Christensen (1929–31) gives a penetrating adverse criticism. The British Ministry of Health (1933) after a careful study in Gerson's clinic by one of its members, finds "no evidence that there would be an appreciable improvement in the results of sanatorium treatment if the diet should be completely adopted. A special kitchen is necessary, the diets cost 30 per cent more than the customary sanatorium diets, and they are too unpleasant for English patients."

INFECTIONS IN MAN, INFANTS AND YOUNG CHILDREN. 1. *General remarks.* Within the last 60 years, the death rate from infectious diarrhea in the first year of life has been greatly decreased by the use of uncontaminated milk. The incidence and severity of respiratory infections have decreased much less. The belief is widely held that proper diet should bring about such a decrease. The analysis already given of animal experiments dealing with the influence of nutrition on infection has indicated: 1. That outspoken deficiencies may lower resistance to infection. 2. That increased feeding of such food-elements as vitamin A to the normal animal may not increase resistance. 3. That the results obtained with one species do not necessarily apply to another species. 4. That very few studies have been made in animals receiving a diet only partially defective. Yet, partial deficiency in man is far more likely to occur than total deficiency. 5. That multiple deficiencies in animals have not been adequately studied. Probably multiple partial deficiencies in man are frequent and important.

Before discussing the several deficiencies which are considered important in causing the loss of resistance to infection in infants, it is necessary to approach the question from a more general point of view. Pettenkofer suspected that there was a relationship between susceptibility to infection and the water-content of tissues. Weigert (1905) showed that the water-content of tissues could be decreased by a diet high in fat and protein. Diets low in these foodstuffs and high in carbohydrate, such as cane sugar, are frequently used by the poorer classes, for feeding infants; Berend (1910) described a series of cases of pneumonia in children who had previously been fed on diets low in fat and

protein and high in carbohydrate. In many of these cases, a very rapid and profound loss of weight was noted at the onset of the illness. Such considerations as these had led Czerny to define an "exudative diathesis," characterized by the occurrence of frequent and severe catarrhal inflammations of the mucous membranes, enlargement of the lymph nodes, eosinophilia, eczema, and, later in life, asthma. Many of the characteristic signs would now be considered allergic in nature. A disturbance in regulation of the water-content of the tissues is the essential feature of the exudative diathesis. Czerny (1913) believed that a diet high in fat was of great importance in preventing and correcting the condition; he also hinted at the importance of the fat-soluble vitamins. Rietschel (1919) and Niemann (1919) reported that diets high in fat confer resistance to pneumonia upon infants. The latter author challenged the dictum of Pirquet, who had stated that carbohydrate may replace without harm, an iso-caloric amount of fat in the infant diet. In the same year, Kleinschmidt (1919) showed that his "butter-flour" mixture could with advantage be fed during an acute infection. More recently, Senff (1932) has called attention to the prognostic importance of "hydrolability. He excludes from his concept the allergic manifestations which Czerny had included in the "exudative diatheses. The chief characteristics of hydrolability, according to Senff, are: 1, sudden changes in the daily weight, especially a marked loss of weight during acute infections or during fasting; 2, marked variations in the excretion of salts, especially alkalis; 3, lack of tissue-turgor; 4, lowered resistance to infections, especially of the respiratory tract; 5, delayed recovery from infections; 6, abnormal febrile reaction to infection, either very high fever or subnormal temperature. Senff, in making a diagnosis of hydrolability, uses a modification of the criterion of Stoeltzner (1925): "Hydrolability is said to exist, if during an infection, the average daily losses are greater than one half of the average daily gains." Otherwise, "hydrostability" is said to exist. In a series of 610 infants, Senff shows that such infections as bronchopneumonia, pyodermia, angina, and otitis media, pursue a much less favorable course in the hydrolabile infant. He suggests, as cure for hydrolability, the feeding of diets rich in "lipoids," especially egg-yolk. He does not give any data showing the result of such treatment, nor does he mention, specifically, the use of vitamins. In 1932, Stoeltzner reported, with details, his success in preventing the development of hydrolability in infants by the use of egg-yolk. Although fat-soluble vitamins may play a rôle in the pathogenesis of this condition, it is important to recognize the fact that the etiology is not understood.

In studying groups of infants of all ages in homes for waifs and orphans, Wertheimer and Wolff (1921) have called attention to the facts long known, that during the first three months of life, infections are rare; after the age of six months, infections are common. The nutritional state has no influence in preventing infections; but is of great importance in determining their course. Adopting the term "Dysergy," introduced by Abels (1924) in studies on scurvy, to mean "Unfavorable response to infections," Wertheimer and Wolff showed that dysergy is characteristic of infants of low birth weight, especially the premature; and of infants with athrepsia, ("dekomposition" of Finkelstein; marasmus of English authors), and even long after apparent recovery from athrepsia. At the time of this report, little use was made in Germany of feedings high in fat, and high caloric diets were not frequent. A number of writers feel that diets somewhat higher than usual in calories may prevent dysergy. Hoeckle (1924) believed that infants under his care, who received about 140 calories per day survived infections much better than infants in other institutions receiving about 100 calories per day. Although the feedings were rich in fat, he used no butter but margarine exclusively. Nassau (1925) presented some important data with regard to the progressive improvement in the health of inmates of the Berlin Home for Waifs, and of the Berlin Asylum for Children. In the following table, "index infectiosus" means the number of infections per infant in 100 days. "Grippe," refers to what is often called in America, "acute upper respiratory infection."

| | 1919–20 | –1921 | –1922 | –1923 | –1924 | –1925 |
|---|---|---|---|---|---|---|
| Number of infants | 1751 | 3039 | 2333 | 2297 | 2058 | 1325 |
| Aver. Gain second 6 mos., grams | 1440 | 1315 | 1160 | 1865 | 2085 | 2770 |
| Aver. Gain year, grams | 3285 | 3075 | 3845 | 4525 | 5190 | 5735 |
| Index Inf., I Quartal of year | 1.0 | 1.2 | 1.3 | 1.1 | 1.1 | 0.7 |
| Index Inf., II Quartal of year | 2.1 | 2.2 | 2.3 | 2.2 | 1.4 | 1.3 |
| Index Inf., III Quartal of year | 2.3 | 2.7 | 2.5 | 2.6 | 2.0 | 1.9 |
| Index Inf., IV Quartal of year | 2.9 | 2.6 | 3.0 | 2.6 | 2.0 | 1.7 |
| Mortality % admission | 15.1 | 12.9 | 13.3 | 13.8 | 9.3 | 7.6 |
| Mortality, per 10,000 days care | 33.5 | 39.8 | 30.7 | 24.9 | 17.6 | 9.6 |
| Grippe: per 100 infants | | | 183 | 153 | 189 | 120 |
| Bronchitis: per 100 infants | | | 66 | 84 | 51 | 54 |
| Bronchopneumonia: per 100 infants | | | 30 | 45 | 9 | 4 |

During the period of this study (1919–1925), no change was made in the technique of isolating the infants from infection; and the type of infant entering the institutions was the same. But the diet was progressively altered: mixtures of milk, flour and sugar, were supplemented earlier and to a greater extent with cereals, fruit juices and vegetables. Moro believed that vitamin C played some rôle in causing the improvement in the health of the infants, because scurvy, which occurred in every seventh infant in 1922, had since disappeared. He stated, however, that other factors than vitamin C must have been involved. So far as appears in his article, cod liver oil was not used.

Levy (1926) found that the course of an infection already present is not influenced by diets rich in fat. He used margarine and no cod liver oil.

The clinical reports so far quoted indicate that faulty methods of feeding produce in the infant no greater susceptibility to infection, but beyond any question lead to a decreased resistance after the infection has already occurred. The effect of faulty nutrition may last a considerable time; a fact which may justify the use of such terms as the "exudative diathesis," "hydrolability," or "dysergy." Acceptable criteria for establishing the existence of these conditions can hardly be said to exist, and the causative factors are by no means established. Among the causative factors in all likelihood are long-continued feeding of diets low in total energy value, in fat, protein, and vitamins.

2. *Vitamin B.* It has been suspected, from experiments with animals, that human dietaries might be deficient in the vitamin B complex. Maurer and Tsai (1932) suggest that such a deficiency can be in part responsible for premature birth, with a resulting decrease of resistance in the children who survive. The careful inventories of maternal diets by Wills and Talpade (1930) prove this more definitely. The latter investigators showed that 24 of 25 mothers in Bombay who gave birth to premature infants, had had diets notably lacking in vitamin $B_1$. No studies have appeared which would indicate that infants consuming diets low in the B complex are liable to infection. For example, infection is not mentioned as a symptom of "relative deficiency" by Dennett (1929).

3. *Vitamin D.* The rachitic infant is stated to be unusually susceptible to respiratory infection. No proof exists for this belief. It is, however, certain that marked rachitic deformities of the chest may aggravate pneumonia in a rachitic infant. The essential difficulty in proving that rickets per se predisposes to infection is that foods lacking

in vitamin D also lack vitamin A. The attempt to prevent and to ameliorate respiratory infections by the use of irradiation with the carbon-arc or the mercury vapor quartz lamp, have been entirely unsuccessful (Barenberg, Friedeman, and Green, 1926; Barenberg and Lewis, 1928). It may be said that the experiments of these investigators were conducted on only a small number of infants; but they were carried out for a very considerable period of time. The infants in the control and in the experimental group were provided with optimal amounts of vitamin D in the form of cod liver oil.

4. *Vitamin C.* Abels (1924) reviewed earlier work on infection and scurvy; and stated his concept of the "dysergy" of scurvy. One characteristic of this dysergy is said to be lack of resistance to infection. It is evident that during shortage of anti-scorbutic food, when scurvy is frequent in the whole population, infections, especially tuberculosis, are of frequent occurrence (Schagan, 1924). A series of infections occurred in 34 of Rosenbund's cases (1923) *before* scurvy became evident. Possibly these infections precipitated scurvy—as did vaccination in the cases reported by Stern (1923). A vicious circle could then arise. It must, however, be remembered that in infants with scurvy, other deficiencies may exist. Bloch (1928) states that the frequency and severity of infections in scurvy is not much greater than that in any other group of hospital cases—with the exception of those with xerophthalmia.

In infants not definitely scorbutic, Meyer (1925) reported that the tendency to develop repeated infections is greatly lessened by the administration of 50 to 100 cc. of orange-juice a day. Reyher (1925) recorded 24 cases of pyuria, of which 38 per cent died and 29 per cent recovered. In 29 other cases, he administered 100 to 200 grams orange-juice and a commercial yeast extract; of these cases 3.4 per cent died and 86 per cent recovered. The second series of cases was studied after the first; the treated and untreated cases were not taken alternately at the same time. Orange-juice undoubtedly contains an active factor other than vitamin C. Debris (1931) thought that lemon juice might in some instances, cure pyuria. He has recently advocated a mixture of all vitamins.

It is clear that infections may be frequent and severe in scurvy, or in the "prescorbutic state." It is not yet known how frequently latent scurvy occurs, nor is it proven that in infants fed on a normal diet, increase of vitamin C will prevent severe infection.

5. *Vitamin A.* Bloch, (1928) who during the late war, had a better opportunity to observe xerophthalmia than any other pediatrician has

had, was impressed with the frequency of severe infections in infants suffering from this condition. He felt that scurvy, on the other hand, does not predispose to severe infection. Between 1911 and 1927, he made the observations here recorded:

| | TOTAL | SEVERE INFECTION | DIARRHEA | NO INFECTION |
|---|---|---|---|---|
| Scurvy | 35 | 9 | 3 | 23 |
| Xerophthalmia | 109 | 68 | 23 | 18 |

Of the 18 cases of xerophthalmia without infection, one had mild conjunctivitis; and the remainder were young infants. We have already noted that severe infection is rare below the age of six months. The 68 cases of xerophthalmia with infection included 15 with pneumonia, 12 with bronchitis, 13 with otitis, 27 with pyuria, 14 with pyodermia. Bloch stated (1931) that children who have recovered from xerophthalmia are liable to an unusually high death rate up to the age of eight years. A high incidence of severe infection, in xerophthalmia, however, is not recorded by all authors: Spence (1930) in England; Blackfan and Wolbach (1933) in America.

Xerophthalmia is an exceedingly rare condition. If deficiency of vitamin A does occur, it must as a rule be only partial. The occurrence of partial deficiency might be determined by analysis of tissues, especially of the liver; by detection of night-blindness; or by showing that resistance to infection was increased by administering vitamin A. Wolff (1932) using the color-test of Price and Carr, found that at birth, the liver may contain little or no vitamin A, especially in premature infants. Thereafter, the quantity rises very rapidly. No criteria yet exist as to what amount of vitamin A in the liver of man should be regarded as normal. Night-blindness in America at least, is extremely rare (Hess, 1933). Jeans (1933), by using a refined technique, has been able to detect poor adaptation to dim light in 20 per cent of older children visiting his clinic, and to relieve this condition with cod liver oil.

The efforts to increase resistance to infection by administering vitamin A do not yet furnish any conclusive proof for the existence of relative deficiency of vitamin A. Erben (1933) reported success from the administration of a concentrated preparation to 37 infants, aged 3 months to 3 years. The respiratory infections were no less frequent, but their course was milder. Barenberg and Lewis (1932), using cod liver oil as source of extra vitamin A, and giving viosterol to controls,

were able to show that infections occurred with equal frequency in both groups.

| GROUP | NUMBER OF INFANTS | INFECTIONS | | | | PNEUMONIA CASES PER MONTH |
|---|---|---|---|---|---|---|
| | | Total | Mild | Moderate | Severe | |
| | | | *per cent* | *per cent* | *per cent* | |
| CLO | 35 | 51 | 37 | 29 | 33 | 2.00 |
| Viosterol | 32 | 43 | 25 | 32 | 42 | 1.14 |

The slight decrease in the proportion of severe to mild infections in the group receiving cod liver oil, is not to be regarded as significant in groups so small as these. It must also be emphasized that the children who had no cod liver oil were receiving milk, which contains vitamin A; expressed in Sherman units per day, those under six months of age had 1500 units; those over six months of age, 3000 units. Three teaspoons of cod liver oil added 7500 units, and six added 15,000 units. Hess, Lewis and Barenberg (1933) continued these studies in the winter of 1932–33 using as sources of vitamin A, halibut liver oil or carotene in quantities equivalent to 20,000 units vitamin A, per day; the control infants received viosterol only. Again, the results were not favorable to the assumption that relative deficiency of vitamin A exists:

| | NUMBER OF CHILDREN | INFECTIONS | | | |
|---|---|---|---|---|---|
| | | Total | Mild | Moderate | Severe |
| | | | *per cent* | *per cent* | *per cent* |
| Vitamin A group | 77 | 245 | 51 | 24.5 | 24.5 |
| Control | 75 | 241 | 59 | 21.8 | 19.1 |

In reading this paper it will be noted that relatively few of the children were under six months of age (approximately 7.3 per cent). No record is available of the early feeding of the children studied. Experiments with animals would indicate that this period is critical (McCoy, 1934). That the feeding in the first few months of life may be of importance in affecting the course of infection in later life, is indicated by observations of Clausen, (1934). In the cases observed by him, the type of infection is classified as *none, moderate,* and *severe.* The vitamin A group includes all infants who received cod liver oil from the third month onwards; and vegetables from the sixth month: (see appended table).

The possible effect of the early feeding of vitamin A is evident. This work may be subject to the criticism that reliance had to be placed upon the clinical histories as obtained.

| 0–6 MONTHS INFECTIONS, PER CENT | | | | | | | 6–24 MONTHS INFECTIONS, PER CENT | | | | | 2–6 YEARS INFECTIONS, PER CENT | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CLO | VEG | Cases | O | M | S | S/Total | Cases | O | M | S | S/Total | Cases | O | M | S | S/Total |
| 0 | 0 | 35 | 40 | 37.1 | 22.9 | 0.38 | 50 | 14 | 40 | 46 | 0.54 | | | | | |
| + | 0 | 27 | 555 | 29.6 | 14.8 | 0.33 | 21 | 9.5 | 86 | 4.5 | 0.05 | | | | | |
| 0 | + | | | | | | 77 | 24.7 | 59.8 | 15.6 | 0.21 | 267 | 32.5 | 55.1 | 12.4 | 0.18 |
| + | + | | | | | | 56 | 41.1 | 55.4 | 3.5 | 0.06 | 124 | 60.5 | 34.7 | 4.8 | 0.12 |

DIET AND INFECTION IN OLDER CHILDREN AND ADULTS. Attempts to decrease susceptibility to infection and to increase resistance by dietary measures, in older persons, have included the modification of the mineral content of the food; and the use of vitamins. We should add that the use of ultraviolet irradiation for this purpose, advocated by Maughan and Smiley (1928, 1929) has been carefully studied in a most critical manner by Doull, Hardy, Clark and Hermann (1931) and has been found quite ineffective.

*Minerals.* Sauerbruch (1924) stated that wounds heal more rapidly in persons receiving a diet with acid ash than in those receiving alkalis. Jarvis (1930) on the other hand, prescribed alkalis for respiratory infections. Friederich (1932) believed that a rapid change from one diet to another is of benefit. None of these authors presented statistical data.

*Vitamin A.* The existence of relative deficiency of vitamin A in older persons remains to be established. Laquer, Wolff and Dingemanse (1928) presented a few observations on the vitamin A content of the human liver. Higher values were found in persons dying of cerebral tumor or trauma than in those with acute infections. The more extensive data of Moore (1932) confirmed this observation. It is not possible to state that the low values preceded the infections: and a number of cases in each series had high values.

A favorable influence upon infection as a result of administering vitamin A, has been claimed by several authors. Green, Pindar, Davis, and Mellanby (1931) administered to 275 women during the last month of pregnancy, a preparation of vitamins A and D ("Radiostoleum").

Of these women, 1.1 per cent developed post-partum fever. Of 275 controls, 4.7 per cent did. Holmes, Pigott, Sawyer, and Comstock (1932) administered one tablespoonful of cod liver oil per day to a *volunteer* group of industrial workers and recorded in them, and in controls not receiving the oil, the incidence of colds:

| | COD LIVER OIL | CONTROL |
|---|---|---|
| Per cent persons without colds | 55 | 32.8 |
| Per cent persons who lost no time | 51.9 | 40.6 |

Donaldson and Tasker (1930) stated that the death-rate from pneumonia in a group of workers at the Crown Gold Mines, Johannesburg, was 8 per cent in those who received either ½ lb. liver per day, or radiostoleum; whereas it was 13 per cent in the control group.

In measles, because of the damage known to occur to epithelium, Ellison (1932) gave 600 alternate cases a concentrate of vitamins A and D. The mortality was 3.7 per cent in the group of 300 receiving added vitamins, and 8.7 per cent in the control group. In 4978 cases of measles in London at the same time, the mortality was 8.1 per cent. Pneumonia and otitis media occurred with equal frequency in both groups. One possible criticism is that the groups occupied separate wards; the argument would have been more impressive if alternate cases on each ward had received the vitamin preparation.

McNeil and McGowan (1913) had observed the repeated outbreak of epidemics of severe respiratory infection in a school for boys near Edinburgh. Better dormitories were built, and heating was installed, without effect. G. M. Findlay found that the food had been boiled for three to six hours in open kettles. After fresh swede-juice and cod liver oil were added to the diet, the epidemics ceased.

Another group of workers has failed to note any anti-infective action of vitamin A. A concentrate equivalent to 400,000 U. S. P. units vitamin A, per day, was administered by Sutliff, Place and Segool (1933) to 509 children with scarlet fever throughout their stay in the hospital. The incidence of otitis media was 9.4 per cent. The expected rate was 11.3 per cent. Cases were not treated alternately. Burton and Balmain (1930) found that vitamin A had no effect in rendering the Dick test negative, either in pregnancy or in patients with scarlet fever.

It is evident that if a relative deficiency of vitamin A exists in only a certain number of persons, and if cases in the experimental and control

group are numerous enough to include by chance equal numbers of persons with the relative deficiency, a small difference in severity of infection should exist between the treated and untreated groups. But if the occurrence of relative deficiency is infrequent, this difference may be so small as to be of no statistical significance. Studies on the carotinoid pigments of the blood and susceptibility to repeated infection lead to the belief that about five or ten per cent of older children *might* be benefited by the use of carotene (Clausen, 1933). Jeans (1933), using a method for testing visual adaptation to dim light, places the incidence of relative deficiency at 20 per cent. It is quite obvious that a crucial test of the anti-infective value of vitamin A or of provitamin A (Carotene), can be made by excluding from observation the large number of persons who cannot have a relative deficiency of the vitamin.

In many published studies on diet and infection, both in animals and in man, use has been made of sources of vitamin which contain many other substances. It is obvious that progress will be more rapid when pure preparations are available.

It is further evident from animal experiment, that vitamin D and vitamin A, in large doses are toxic. The toxic effect varies not only with the dose administered, but with the species, and with other constituents of the diet. It is accordingly of the utmost importance to use standardized preparations of the pure vitamins, to vary nothing else but the vitamins, in the control and the experimental groups, and to state in detail the nature of the basic diet.

General conclusions. Susceptibility to infection is not as a rule affected by diet. Resistance to infection, on the other hand, may be greatly reduced by deficient diet. A deficiency in the diet of vitamins A and C appears quite definitely to lower resistance to infection. In certain cases, a lack of the vitamin B complex may also do the same thing. A lack of vitamin D cannot be said to have a proven effect in lowering resistance. It seems probable that the existance of a partial deficiency of vitamins may result in loss of resistance to infection, though this cannot be said, from the present evidence, to have been clearly established.

The evidence discussed in this paper points to certain lines for future investigation. In the study of the influence of diet upon resistance to tuberculosis, we need to discover methods of producing in experimental animals, a chronic form of the disease like that in man. In the study of human tuberculosis we need a more careful analysis of the effects of minerals, in particular, of sodium chloride, and of the acid and base values of the diet; and of deficiencies of vitamins A and C.

We need a search for the existence of anti-infective substances other than the ones already recognized in natural foodstuffs, e.g., fruit juices. We need the use of highly purified vitamins in experiments designed to study the question of partial deficiencies. We need to use, and perfect, chemical methods for analysis of tissues and foodstuffs. We need a more careful study of tissue reactions in animals suffering from food deficiencies and infections, and finally more carefully planned experiments dealing with partial and multiple deficiencies.

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# THE PHYSIOLOGY OF THE PHOSPHOLIPIDS

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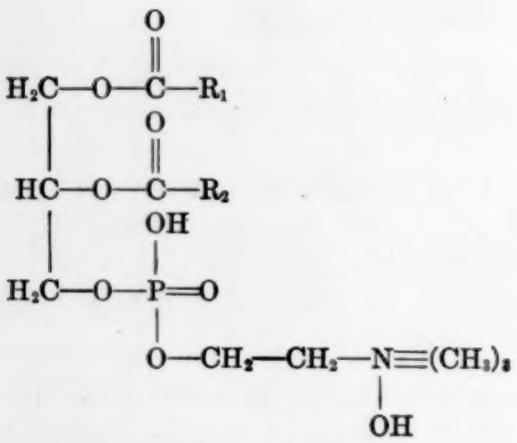
The phospholipids are ever-present constituents of every living thing. First detected in 1812 as a fat-soluble fraction of the phosphoric acid of human brains (307), they have since been found in all cellular material in which they have been properly looked for. It may be inferred therefore that the phospholipids play some fundamental rôle in the structural make-up and the vital activity of the cell. In this review an attempt has been made to outline the present state of our knowledge concerning the physiological behavior of the phospholipids and the functions which they perform. The discussion will be limited, for the most part, to information bearing on the phospholipids of the animal organism.

CHEMISTRY. The phospholipids are definite chemical compounds containing both fatty acid and phosphoric acid.

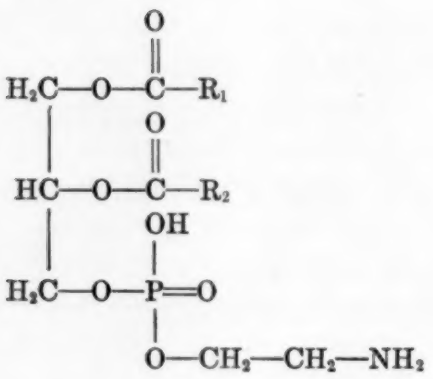
*In animals* three types are found: 1, the lecithins—substances consisting of two fatty acids, glycerol, phosphoric acid, and the nitrogenous base choline; 2, the cephalins—substances similar to the lecithins except for the substitution of aminoethyl alcohol for choline;[1] 3, the sphingomyelins—substances allied to the lecithins in that they contain phosphoric acid and choline, and to the cerebrosides in that they contain the nitrogenous base, sphingosine, and, in part, $C_{24}$ fatty acids. The formulae for these three phospholipids are given on the following page.

Each of these three groups contains several members differing from one another in the length of the carbon chain and the degree of unsaturation of the fatty acids present in the positions indicated by $R_1$ and $R_2$. In the case of the lecithins and cephalins, the question of the identity of the fatty acids which may be present in the molecule will

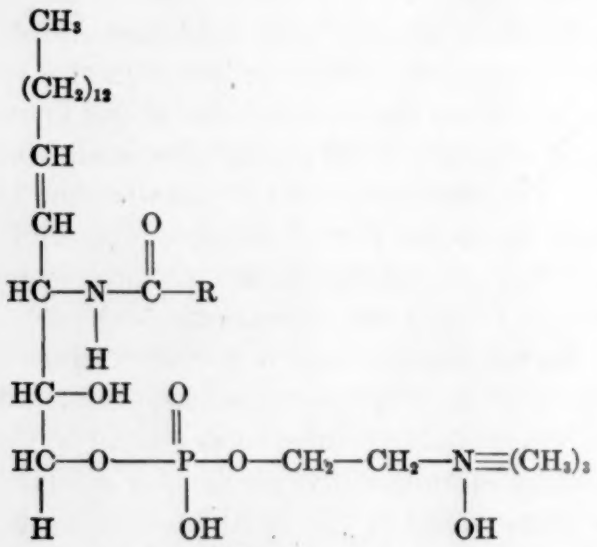
[1] There is still doubt as to the constitution of the cephalins. As isolated from animal tissues and purified by the accepted methods, the cephalin fraction, on analysis, shows a poor agreement with the formula assigned. Whether the fault lies in the presence of contaminating split products as thought by Levene (158) and MacLean (174), or in the presence of some unidentified constituent of the molecule, is still uncertain.



Lecithin



Cephalin



Sphingomyelin

come up for discussion later on. As for the sphingomyelins, three are known to exist, corresponding to the three fatty acids, lignoceric, nervonic and stearic (298).

As the formulae indicate, the possibility also exists of there being two groups of lecithins and cephalins, differing in the point of attachment of the phosphoric acid to the glycerol. There is reason to believe that such $\alpha$- and $\beta$-lecithins and cephalins do exist in both plants and animals (298, 321).

The phospholipids present in the tissues of animals consist for the most part of lecithins and cephalins. The sphingomyelins are known to be present in many organs—brain and nervous tissue especially, adrenals, kidneys, liver, spleen (298); erythrocytes (47); and heart (139).

However, in comparison with lecithins and cephalins, the amount of the sphingomyelins is certainly small and, for lack of a suitable method, next to nothing is known as to their physiological behavior and probable significance.

METHODS OF DETERMINATION. 1. *Lipid P.* In by far the great majority of investigations concerned only with the amount of phospholipid, the method employed has been to determine the amount of lipid P present. Various procedures have been used to secure complete extraction of lipids from the tissues and at the same time to avoid extraction of non-phospholipid P. In some of the older work the tissue was hashed and dried and then extracted with ether. But ether does not extract all of the lipid from dried tissues unless the latter have previously been treated with alcohol (243). The most frequent procedure has been to extract with alcohol or a mixture of alcohol and ether, evaporate the extract to dryness and then reëxtract the residue with dry ether. Such a procedure gives too high results. As shown by Le Breton (154) approximately 20 per cent of the phosphorus in the ether extract is not true phospholipid P; that much is removed from the phospholipids by precipitation of a saline emulsion of the latter by acetone. In an effort to avoid this fault of ether, several other fat solvents—methyl alcohol (278), chloroform (8), benzene (190) and petroleum ether have been tried. While these probably do exclude some of the contaminating phosphate-containing substances soluble in ether, it has been shown (8) that chloroform, at any rate, also dissolves about 18 per cent more P from the dried alcohol extract of liver than corresponds to the phospholipid content.

2. *Acetone precipitation.* The phospholipids are practically insoluble in acetone; neutral fat, cholesterol and cholesterol esters are soluble in acetone. This difference makes it possible to secure a satisfactorily complete precipitation of the phospholipids, free from fat and cholesterol, by the addition of acetone to the ether, petroleum ether or chloroform solution of the total lipids of blood or tissues. However, as Nerking showed years ago (216), precipitation is complete only if some salt such as $MgCl_2$ is added to the acetone. To ensure complete freedom from neutral fat, at least one reprecipitation of the phospholipid should be carried out (8).

After isolation of the phospholipid, the amount may be determined in various ways. Weighing is open to the objection that an unknown amount of $MgCl_2$ has been carried down by the phospholipid. Small amounts may be oxidized by the Bloor chromic acid procedure (28); to

the extent that the phospholipid is contaminated by oxidizable "carnithine" substances (173), the Bloor procedure will give too high results. To avoid such errors, the phospholipids may be saponified and the fatty acids determined by weighing, by titration (8, 185), or by oxidation.

PROPOSED FUNCTIONS OF THE PHOSPHOLIPIDS. Since the ultimate significance of the various observations to be discussed in this review lies in their bearing on the question of the functions of the phospholipids in the organism, a brief outline of the various proposed functions will be given at this time. Of these there are three which can be stated in concrete terms:

a. *The metabolic theory* holds that the phospholipids—or at least the lecithins—are intermediary products in the metabolism of fats and the fatty acids. This theory originated with Loew (161) in 1891 and since that time has been widely accepted. In its present day form, which also incorporates the Leathes theory of desaturation of the fatty acids in the liver as a primary step in their oxidative breakdown in the tissues, the theory reads about as follows: Neutral fat, either from the fat stores, as in the case of fasting, or from the alimentary tract, is taken up by the liver where it undergoes two processes: desaturation, and transformation into phospholipid. Which of these steps occurs first is not always stated although Kennaway and Leathes (134) early pointed out that the high degree of unsaturation of liver fatty acids applied to both the phospholipid and neutral fat fractions. In any case, the highly unsaturated phospholipid passes out into the blood stream, and is carried to the other tissues where the fatty acids are burned. As conceived by Loew, the phospholipids thus constitute a machine for the transporting and burning of the otherwise water-insoluble and stable fatty acids. Other than that they are miscible with water, there seems to be no substantial reason for believing that the phospholipids pass through the capillary endothelium any more readily than the neutral fats. Indeed it has been shown just recently that the phospholipids appear to be held back in the capillaries to about the same extent as the plasma proteins (186). That the capillary wall is not impermeable to neutral fat is strongly indicated by the fact that we have no reason at present for believing that food fat, which reaches the blood stream in the neutral form, must be changed over into phospholipid in order to get into the adipose tissue cells where it is stored. Nor have we any direct evidence at present that the presence of the phosphoric acid in the same molecule with the fatty acids has any catalytic action on the rate of oxidation of the latter, as has been so frequently suggested.

b. *The oxygen transport theory* holds that the phospholipids, by virtue of the unsaturated C=C linkages in their constituent fatty acids, serve as a mechanism for the carrying of oxygen within the cells. By the same token, the unsaturated bonds can be considered as potential hydrogen acceptors as well as oxygen acceptors. *In vitro* unsaturated fatty acids can be either oxidized or reduced. Although this theory was first advanced in 1903 by Koch (142) and has since been subscribed to by a number of workers (92, 196, 302) there is, as yet, practically no experimental evidence in its favor. The essence of the theory, of course, is the presumed reversibility of the oxidation (or reduction) of the double bonds under physiological conditions. This has never been demonstrated. On the other hand, it has been shown that phospholipids from eggs or mammalian tissues do oxidize under simulated physiological conditions, with disappearance of the unsaturated bonds (222). Thus, it was demonstrated by Thunberg (299) and by Warburg (310) that egg lecithin will consume oxygen in the presence of ferrous iron, the oxygen consumed being somewhat more than twice that required to account for the formation of two hydroxyl groups at each double bond. No $CO_2$ is produced. More recently it has been shown that glutathione (115) and other sulfhydryl compounds such as thioglycollic acid and cysteine (200) are able to accelerate the oxidation of unsaturated fatty acids, both in phospholipids and in the free state.

The experiments of Meyerhof (200) are especially interesting. Finely hashed and repeatedly washed frog muscle, on addition of sulfhydryl compounds will consume several times as much oxygen in the same period of time as will the hashed muscle alone. This excess oxygen is much more than can be accounted for by the oxidation of the SH group to the disulfide. The same increased oxygen consumption was found with a dry powder of frog muscle previously extracted with cold alcohol. Extraction by hot alcohol and then by ether abolishes $O_2$ consumption. It was then found that egg lecithin and also the acetone-insoluble fraction of the alcohol-ether extract of beef heart and beef muscle consume oxygen in the presence of sulfhydryl with a decrease in their iodine-binding capacity, i.e., in the double bonds.

Now, these experiments were carried out on water or cold alcohol extracted muscle or on emulsions of phospholipids. If unwashed frog muscle was used, there was a vigorous oxygen consumption but no change whatsoever in the unsaturation of the muscle lipids. Oxidation of the water-soluble extractives (sugar, lactic acid, etc.) was thought to have been responsible for the $O_2$ consumption. It is possible to infer that in

the unwashed muscle, oxidation of the double bonds had actually occurred but that reduction had followed immediately, the oxygen being handed on to the sugar. Meyerhof himself hinted at such a process but considered it hardly worth while speculating about it.

Further evidence of a possible catalytic action of the unsaturated phospholipids in oxidations is afforded by the observations that addition of lecithin (but not of cephalin) increases the consumption of $O_2$ by a suspension of dried heart muscle in phosphate buffer containing potassium succinate (302). Extraction of the heart muscle powder with fat solvents reduced or completely abolished the $O_2$ consumption, and, according to Kakiuchi (131), the reduction parallels the extent to which the mitochondria are destroyed.

The belief that the phospholipids of the cell are in part at least concentrated in the mitochondria is of long standing (232) and is supported by such evidence as the solubility of mitochondria in ether, chloroform, and glacial acetic acid, insolubility in acetone, characteristic staining reactions after chromic fixation—attributed to the formation of oxyacids (196),—and parallelism between changes in mitochondria and phospholipid (196). Not all, however, attribute to the mitochondria the function of acting as oxygen transfer agents or centers of oxidation. They have also been regarded as centers of protein synthesis (238) and of enzyme production (116).

c. *The structural theory* holds that the phospholipids by reason of their peculiar physico-chemical properties—solubility in fats and yet water miscibility; lyophilic properties; low surface tension; high degree of polarity of the molecule—are important elements in the constitution of protoplasm and in the construction of the membrane enveloping the cell (153). In other words, the phospholipids fall into the same class with the proteins in being fundamental units in the structure of living matter. Needless to say, this does not mean that either the phospholipids or the proteins are inert materials, a mere shell in which life goes on; rather, it means that they are involved neither as fuel nor as purveyors of fuel in any specific energy-producing reaction of living matter.

Direct experimental evidence in support of the structural theory is very scarce. It is based to a great extent on deductions made from permeability studies, especially on the red blood cell (94). And it is in the red cell that we have the best direct evidence that the phospholipids are one of the components of the cell structure. Here we have a cell which has, at most, a small oxygen consumption and therefore has no appreciable fat catabolism, which contains no mitochondria (65), and

yet has a very sizeable phospholipid content much—if not all—of which is present in the stroma (47, 107).

The question of the existence of a lipid membrane has developed a new angle as a result of the observations of Hansteen-Cranner (105, 106) that plant cells contain water-soluble phospholipids which can alternately become diffusible or non-diffusible depending upon the absolute and relative concentrations of inorganic salts in the surrounding medium. The permeability of the cell to other substances such as the natural pigment of beets varies directly with the diffusibility of the water-soluble phospholipids. One important implication in this work is that many of the physical and chemical properties of the phospholipids as they are ordinarily prepared are not those of the native substances but have arisen as a result of denaturation incident to preparation.

THE DISTRIBUTION OF THE PHOSPHOLIPIDS. a. *Comparative content of various organs, tissues, and fluids.* Just as each organ of the body has its own peculiar histological structure and its own peculiar function or functions, so, too, each has a more or less characteristic phospholipid content. The following data, taken from the work of Bloor (25, 26, 27), illustrate the approximate differences in the phospholipid (lecithin + cephalin) contents of various organs of the beef. The amounts per 100 grams of fresh organ are: brain, 4.58; liver, 3.06; pancreas, 1.86; kidney, 1.62; lung, 1.25; heart muscle, 1.64; jaw muscle, 1.06; diaphragm, 0.76; neck muscle, 0.63; round muscle, 0.42.

In the case of the brain and nervous system, the two parts, the grey and the white matter, differ appreciably in their phospholipid content. Thus, according to Smith and Mair (274) the total lipid P content of white matter is 1.5 times that of the grey matter; according to Koch (143) the corpus callosum, which is pure white matter, contains 8.7 per cent of phospholipid while the cortex, largely grey matter, contains 3.9 per cent, based on moist weight. It is interesting to note that the cerebrosides, which are close allies of the phospholipids, especially the sphingomyelins, are believed to be solely contained within the white matter of the nervous system, indeed within the myelin sheath. Koch (143) proposed that the amount of cerebroside in mixed grey and white matter, such as cortex, be used as a measure of the percentage of white matter present. As is to be expected from its relatively greater proportion of white matter, the spinal cord exceeds the brain in phospholipid content (216). Whether the large amounts of phospholipids, cerebrosides and cholesterol in white matter serve as fuel or as an insulating coat to the conducting axis-cylinder is not known; the latter appears the more probable.

An attempt was made by Gorodisskay (97) to correlate the known function of different areas of the cerebral cortex with their chemical, and especially lipid, composition. While the individual variations were rather great, there is a definite indication that the motor and sensory areas are richer in total lipid, phospholipid, and cholesterol than the association areas.

It is evident from the data given above that muscles differ markedly in their content of phospholipid, heart being highest and the leg muscles lowest. Reasoning from the fact that in beef animals raised for slaughter, the legs muscles are probably inactive much of the time while heart and jaw are almost continuously active, Bloor (26) has concluded that the phospholipid content of the muscles increases in the same order as their activity. Others (76) have pointed out that the phospholipid and cholesterol content of muscles may be correlated with their resistance to fatigue, slowly contracting red muscle (semitendinosus in the rabbit) being richer in phospholipid than the more rapidly contracting and more easily fatigued white biceps femoris (278). In some instances, smooth muscle is richer in phospholipid than skeletal muscle; in other instances such is not the case. In the beef, both uterine muscle and the retractor penis were found to be richer in phospholipid than leg muscle (64); in the cat no significant difference was found between the smooth muscle of the intestinal tract and cross-striated muscle from the hind leg (261). In the heart, the percentage amount of phospholipid apparently differs from one chamber to another; the two ventricles—despite the approximate 5-fold difference in work done—have about the same lipid P content, but the left auricle usually and the right auricle practically always have a lower content than the ventricles (315). The most notable difference, however, is between the conducting mechanism and the muscle of the heart, the lipid P contents of the Tawara bundle and muscle being 0.42 per cent and 0.68 per cent respectively; the cholesterol contents, on the other hand were the same (319).

As compared with the various organs, the fluids of the body, in the normal animal, have a low phospholipid content. In the case of blood it is of course necessary to differentiate between plasma, red cells, and white cells. As will be brought out presently, the phospholipid content of plasma depends upon dietary and other conditions to a much greater extent than is true of the cellular elements of both blood and tissues. The red cells of beef have an average phospholipid content of about 0.35 per cent (calculated from lipid P (175)), that is, even lower than round muscle. The phospholipid content of the white blood cells of both

humans and dogs is roughly twice that of the red cells (38, 39). The phospholipid content of lymph in the fasting dog has been found to range between 0.16 and 0.27 per cent, averaging 0.20 per cent (72). The evidence that the capillary membrane has a limited permeability to phospholipid as well as to protein (186) raises the important question of the origin of the phospholipids of the lymph. A fasting level of 0.20 per cent is about that which might be expected in the blood plasma. To what extent the phospholipid participates in the observed increase in total fatty acids of lymph during fasting (240) is not as yet known. The low phospholipid content of blood and lymph as compared to the tissues has an interesting bearing on the question as to whether or not phospholipids are the means by which fatty acids are carried from the bloodstream into the tissues. Since the gradient is from tissues to blood, if

TABLE 1

*Grams of lipid phosphorus per 100 grams of fresh tissue*

| | LIVER | KIDNEY | LUNG | MUSCLE |
|---|---|---|---|---|
| Dog | 0.145 | 0.128 | 0.099 | 0.053 |
| Rabbit | 0.142 | 0.122 | 0.096 | 0.039 |
| Guinea pig | 0.148 | 0.124 | 0.135 | 0.053 |
| Pigeon | 0.143 | 0.157 | 0.107 | 0.093 |
| Eel | 0.127* | 0.120 | ..... | 0.036 |

* Hepato-pancreas.

the phospholipids are a transport mechanism, then the bulk of the tissue phospholipid must be rendered non-diffusible in some way. Spinal fluid has been found to be practically lipid-free under normal circumstances, the phospholipid content being given as 1 mgm. per 100 cc. (141).

Of the secretions, bile (164) and milk (114, 301) are definitely known to contain phospholipid. The amount is small and it is questionable as to whether the phospholipid in these secretions is a functional or merely a fortuitous constituent. It has long been held, of course, that the phospholipids in bile enhance its ability to emulsify and therefore to promote the absorption of fats. It is noteworthy that the phospholipids of milk largely stay in the buttermilk on centrifuging (16, 114), although some is certainly carried over with the butter fat (236, 317).

b. *Comparative phospholipid content of various animals.* In table 1 are given the average values for the lipid P content of several organs in

a number of different animals as found by Mayer and Schaeffer (191). On the basis of these data the authors concluded that the phospholipid content of any given organ is a characteristic of that organ regardless of the species of the animal. Interpreted liberally, as it undoubtedly was meant to be, that statement can be accepted as correct. But there are exceptions to the rule. The most notable exception is striated muscle which, as the above data show, varies to a greater degree from one animal to another than is true of the other tissues and which, as shown by Bloor (26), varies greatly from one muscle to another in the same animal. And yet, a comparison of the lipid P content of striated muscle from the turtle, frog, fish, mussel, snail, and crawfish showed, in all but the mussel, the same value of about 0.032 per cent; the value for mussel, 0.051 per cent, was practically the same as that given for the rabbit, 0.054 per cent (292). A comparison of the oxygen consumption *in vitro* of these muscles with the phospholipid, protein or ash content showed an utter lack of any semblance of a correlation (292). The similarity in the phospholipid content of the same organ in animals with such different metabolic rates as the rat and beef is, in itself, sufficient evidence that the *in vivo* oxygen consumption per unit of organ weight cannot be a *simple* function of the phospholipid content. According to the data of Bolle (34) the amount of phospholipid found in bone marrow differs markedly from one species to another, the extreme values being 1.165 grams per 100 grams of fresh marrow in the cat and 0.316 per cent in the horse. Data on the phospholipid content of one or several tissues of the following animals may be found in the papers listed: man (191); dog (52, 191, 235, 243); cat, (216, 261); rabbit, (52, 110, 191, 207, 216, 224); beef, (25, 26, 27, 235); guinea pig, (52, 191, 249); horse, (124).

PHYSIOLOGICAL FACTORS AFFECTING THE PHOSPHOLIPID CONTENT OF TISSUES. 1. *Dietary conditions.* As a phase of their extensive study of the distribution of tissue lipids, Mayer and Schaeffer (191) investigated the effect of fasting and of feeding diets rich in either carbohydrate or fat on the phospholipid content of the various organs in a number of different animals. Their results led them to conclude that the percentage amount of phospholipid in an organ is remarkably constant, regardless of the dietary conditions of the animal. Now, as compared with the variations which are to be found in the fat and glycogen content of various tissues, the variations in the phospholipid content are indeed very limited. But if the comparison is made with other cellular constituents, notably the total protein and the water, there is no doubt that the

phospholipid content of *certain* organs, at any rate, is subject to very appreciable changes. The correlation of the direction and magnitude of these changes with the factors which bring them about ought to give us some insight into their functional meaning.

*Fasting* can now be said to produce unmistakable changes in the phospholipid content of certain organs, though not of others. In the organs affected, differences are found in the extent and direction of the change. Thus, Cahn and Bonot (52) found that in dogs fasted until the body weight had decreased by 30 per cent, the values for lipid P, referred to the weight of dry fat-free tissue, were higher than normal in the liver, lungs and—probably—the kidneys, essentially normal in the brain and intestine, and about 20 per cent below normal in the muscles. The data on guinea pigs and rabbits, also fasted until a 30 per cent loss in weight had occurred, were in most cases too few to be decisive; however, the decrease in the phospholipid content of striated muscle was definitely established for the rabbit as well as for the dog. A still more striking instance of a decrease in the phospholipid content of striated muscle during fasting is that reported by Greene (100) for the salmon. During its migration from the ocean up the coastal rivers, the salmon does not eat throughout the entire 4 to 5 months which may be required for the trip. In salmon caught at the mouth of the river, the lateral muscle had a phospholipid content of 1.18 grams per 100 grams of moist fat-free muscle; in salmon taken at a point 700 miles from the ocean, the muscle contained only 0.39 per cent of phospholipid. The fat content decreases from 15 per cent to 2 per cent. In contrast to the above findings, no appreciable change occurs in the phospholipid content of the skeletal muscle of the rat during a fast of from 3 to 14 days (269). In the same animals, however, the phospholipid in the liver was found to be consistently above the control level, the average increase being 22 per cent when referred to a moist fat-free basis (268). A *decrease* in the amount of phospholipid in the liver of the rat during fasting has also been observed (205) and why an increase should occur in one instance and decrease in another is still unexplained. In the frog, a fast of 7.5 months caused no change whatsoever in the phospholipid content of the dry tissue, although that of the moist tissue, due to the increase in water content, decreased by 28 per cent (149). When the fast was prolonged to 15 months, then the phospholipid content of the dry tissue was found to be only about $\frac{1}{2}$ of the normal value (150). In the blood, the level of phospholipid has been found to decrease in the dog (160), pigeon (208), and rat (284) but to increase in the rabbit (121).

The *type of diet fed* also has an effect on phospholipid content, although in this case the only organs which can definitely be said to be affected are the liver and the blood. And in these, acute changes can be clearly observed only when rather extreme measures are taken such as the feeding of large amounts of fat or carbohydrate.

In the livers of animals which have been fed on ordinary diets, an increase in the phospholipid content can be produced only if a very large amount of fat is fed at a single meal. Artom (7) (9) gave dogs as much as 30 grams of horse fat per kilo of body weight and found that the liver phospholipid increased progressively up to a maximum of 130 per cent of the normal at the 5th hour and then fell to the normal level by the 10th hour. In cats a diet of beef kidney was observed to give a higher content of phospholipid in the liver than an exclusive diet of beef muscle (261). Similarly rats fed on a diet very low in fat have about 25 per cent less phospholipid per 100 grams of fat-free liver than have rats fed on diets rich in fat (269).

It is in the blood that one can most readily observe consistent and unmistakable changes in the phospholipid content in response to a change in the diet. However, here too the response varies from one type of animal to another and even from one individual to another. While in man (177, 223, 312) and in the dog (19, 122, 160, 233, 306), the administration of a single large feeding of fat generally causes a pronounced increase in both the total fat and phospholipid of the blood plasma within a few hours, in herbivorous animals, such as the rabbit and cow, an acute alimentary lipemia with an accompanying increase in phospholipid is not readily induced. Presumably the rate of absorption of fat by herbivorous animals cannot readily be made to exceed the rate of assimilation.

However, in both carnivorous and herbivorous animals, the level of the plasma phospholipid (and total fatty acids and cholesterol) can be raised or lowered appreciably by increasing or decreasing the fat content of the diet. Indeed, this chronic effect of diet, in contrast to the acute effects of a single feeding, are much more pronounced in the herbivora, such as the rabbit, than in the dog. Thus, Bloor (30) has shown that alternating the dog between low and high fat diets caused, on the average, a shift of about 30 per cent in the plasma phospholipid, while in the rabbit the level shifted from as low as 10 mgm. per cent on the low fat diet to as high as 190 mgm. per cent on the high fat diet. A similar, though not so pronounced, variation in the plasma phospholipid level on changing the fat content of the diet was found in the cow (176). The

change in the post-absorptive level of plasma phospholipid can be produced in the dog by a single overfeeding with fat or carbohydrate (31). The increase may be as much as 40 per cent above the initial level and usually disappears in about 3 days.

There is still considerable doubt as to whether the amount of phospholipid in the red blood corpuscles as well as in the plasma changes with diet, and especially during alimentary lipemia. Bloor (19) observed that the phospholipid content of the corpuscles increased even more than the plasma after the feeding of a dose of olive oil to a dog and put forward the suggestion that the corpuscles were actively involved in the synthesis and transport of phospholipid in the body. While at least one other worker (32) has observed a similar increase in corpuscle phospholipid during fat absorption, others have been unable to do so (122, 306, 312). Bloor, himself, in a later paper, remarked that he was not always able to repeat his earlier findings (22). No explanation for these differences in the behavior of the corpuscles has yet been offered. Recently, evidence from an entirely different angle has appeared in support of the suggestion that the corpuscles are engaged in phospholipid transport. Artom (10, 11) has observed that on feeding or injecting iodized fat, the phospholipids of the red cell not only contain iodized fatty acids but actually contain about 21 per cent of the total lipid iodine present in the cell. However, the actual amount of iodized fatty acid present (8 mgm. per cent) was very small in comparison with the total phospholipid content and did not diminish even at the end of 44 hours of fasting. Both of these facts were recognized by Artom but he felt that their significance was greatly outweighed by the actual demonstration that ingested fatty acids can be found combined as phospholipid.

The simplest and most widely accepted explanation of the changes which can be induced in the phospholipid content of the tissues and the blood by these various dietary measures such as fasting or fat feeding is that they are the response to an increased catabolism or transport of fat. In other words, they are regarded as the natural result of the involvement of phospholipids in the intermediary metabolism of fat. Where there is a decrease in phospholipid, as in the muscles of some fasting animals, then the rate of combustion is assumed to have exceeded for a time the rate of diffusion from the blood; where there is an increase, as in the liver during fasting and after fat feeding, and in the blood plasma after fat feeding, then the rate of synthesis of phospholipid is assumed to have exceeded the rate of combustion. Thus it is the great merit of the metabolic theory that it is capable of explaining an increase, a de-

crease, or no change at all. But, as we shall see presently, there are many reasons for doubting that this explanation, simple though it is, is really the correct one.

Quite apart from its explanation, the question arises as to the origin of the increased amount of phospholipid which is present in the blood stream for hours or even days after the ingestion of a large amount of fat. Aside from the suggested synthesis within the red blood cells, two sources seem especially likely—the liver and the intestinal mucosa. That the liver is able to synthesize phospholipid from fat was claimed years ago by Reicher (233) on the basis of perfusion experiments on excised livers. More recently, other experiments on intact animals have led to the same conclusion (155). Thus, Nedswedsky and Alexandry (213), using London's (163) technique for obtaining blood samples from arteries and veins of unanesthetized dogs have found that blood leaving the liver contains as much as 23 per cent more phospholipid (lipid P) than that entering the liver via the portal vein.

There is at present little to be said in favor of the suggestion that the phospholipid of the blood may have its origin in the intestinal mucosa. However, it is known that in the mucosa there is an active phospholipid metabolism during fat absorption (260), the interpretation being that the phospholipids act as intermediary products in the resynthesis of fatty acids to neutral fat. Now, although no significant changes in amount could be observed, it cannot be denied that some phospholipid may have escaped into the blood stream or lymph and have been immediately replaced by synthesis. Furthermore, Eckstein (72) has observed slight but certainly not negligible increases in the phospholipid content of the lymph during the absorption of fat.

The phospholipids in animal tissues can certainly not be considered as a stored material even though they may be, in part, intermediary metabolites. There are, however, two well-established instances in which the phospholipids *are* accumulated as stored material. One of these is the egg (214). About 10 per cent of the weight of the hen's egg consists of phospholipid—a mixture of lecithins and cephalins (129, 280, 298). During incubation very little change in phospholipid content occurs during the first 10–12 days; thereafter, an active catabolism sets in so that on the 20th day somewhat over 40 per cent (129) and at hatching approximately 70 per cent of the phospholipid has disappeared (229). Two very different interpretations have been placed upon the disappearance of phospholipid from the hen's egg during the latter half of the incubation period. Plimmer and Scott (229) believed that the primary

purpose of the large store of phospholipid was to serve as a source of inorganic phosphate for the formation of bone and they accordingly regarded the period of active phospholipid breakdown as being coincident with the period of bone formation. Hanes (104) offered about the same explanation of the decrease in the phospholipid content of the chick's liver between the 14th and 21st day and attributed the accumulation of cholesterol esters to esterification of the freed fatty acids with cholesterol. But, according to Jost and Sorg (129), the phospholipid of the egg is a real intermediary in the metabolism of the fat also present in the egg. They found that neither phospholipid nor fat was burned to any great extent during the first 10–12 days and that, during the next 6 to 8 days, both disappeared to about the same extent—42 per cent of the initial amount present. Coincident with the decrease in phospholipid there was a drop in the degree of unsaturation, presumably because the more unsaturated phospholipids are used up to a greater extent than the more saturated ones.

The other instance in which phospholipid is certainly a stored material is in the seeds of many plants. During germination the amount of phospholipid decreases (108, 126). In the bean (126) there is a very interesting change in the proportion of the various phospholipids as the development of the young plant progresses. Whereas the cotyledons contain mostly lecithins and cephalins and about 3 to 4 per cent of magnesium phosphatidate, the growing plant contains less of the former and more of the latter. The leaves contain only about 7 per cent of the phospholipid as lecithin and cephalin, the remainder being calcium phosphatidate (58). It has been tentatively suggested that the magnesium phosphatidate of the cotyledons represents a store of magnesium for chlorophyll formation, calcium replacing the magnesium in the green leaf (126).

2. *Synthesis of phospholipid: Absorption from the intestine: Influence of feeding or injecting phospholipid.* There is no doubt that, if provided with the raw materials, the animal organism is able to synthesize phospholipid. By a strange coincidence the first proof of this fact was established independently by two groups of workers at the same time and by the same experiment. In one case (87) ducks, and in the other (178) hens were fed over a period of several months on diets low in phospholipid. In each case the eggs produced by these fowl contained more phospbolipid than was taken in with the food. More recently, the excellent growth of rats on synthetic diets containing fat but not phospholipid has demonstrated that mammals too can synthesize phospholipid at will (48, 49).

To what extent the synthesis of phospholipid is impaired by the absence from the diet of linoleic and linolenic acid, and to what extent, if such be the case, the impairment in phospholipid synthesis is responsible for the poor growth and health of animals on such diets (48, 49, 50), must still be regarded as an open question. The phospholipid content of animals raised on diets deficient in unsaturated fats is entirely normal but one cannot conclude from this that the rate of synthesis of phospholipid has not been a limiting factor in the rate of growth.

Attention may be called at this time to the remarkable observation that when grown on a cystine medium *B. coli* grew slowly and were found to contain only a trace of phospholipid, whereas on an alanine medium growth was good and a large amount of phospholipid was found (73). The existence of living cells free of phospholipid (of one kind or another), is so unique that further study along these lines is badly needed.

Several investigators (33, 60, 230, 256) have found that "lecithin" is readily hydrolyzed by the digestive lipases. This being the case, and in view of the inability up to the present to demonstrate absorption of neutral fat from the intestine, it would seem unlikely that any intact phospholipid would be absorbed from the intestinal tract. Yet the fact of the matter is that numerous workers have claimed that the feeding of phospholipids increases the phospholipid content of the brain (37, 237, 246, 257), of the liver (91, 211, 237), and of various other organs (37, 75, 91, 237). Other workers, however, have reported negative results (191, 207, 211, 254). A further study of this question is needed.

Recently, Pasternak and Page (227) have reported the results of an extensive study of the possibility of influencing the phospholipid content of the various organs of the body by the intravenous injection of brain cephalin. Two procedures were tried—repeated injection over a period of about a month, and single injections of various amounts, with analysis of the organs at various times after injection. The repeated injection of 0.1 to 1.5 grams of cephalin per kgm. of rabbit had no demonstrable effect on the phospholipid content of brain, liver, heart, or plasma. A single large dose of 1.4 to 3.9 grams (1.5 gm. kilo) of cephalin did not affect the composition of the brain or heart but did temporarily increase the phospholipid content of the liver and plasma. In the liver, the increase from the normal of 2.7 per cent up to as high as 4 per cent lasted for less than 12 hours; in the plasma, the increase persisted for several days.

What happens to this injected phospholipid is not known. In some cases there is an increased excretion of phosphoric acid in the urine, indicating that hydrolysis had occurred (227); there was also an accumula-

tion of fat in the liver after the phospholipid increase had disappeared. That the injected phospholipid is not readily burned is indicated not only by the fact mentioned above that it persists in the blood stream for many days but also by the proof that it does not lead to an increased heat production or decreased R. Q. (101). In view of the existence in various tissues of enzymes which readily split off the phosphoric acid from the phospholipids (6, 135, 136, 286, 297, 308), and also in view of the evidence that the amount of phospholipid in tissues is kept rather constant, it seems not unlikely that, if any of the injected phospholipid does permeate the capillary wall and reaches the tissues, it is immediately hydrolyzed. There is, however, some question as to whether or not phospholipid does readily permeate the capillary wall; indeed one can explain the accumulation in the liver but not in other organs by the fact that in the liver the blood passes into open sinuses and therefore comes into direct contact with the liver cells (247).

3. *Influence of age on phospholipid content.* In both rats and mice the percentage amount of phospholipid in the entire animal changes very considerably in the course of post-natal development. When calculated in terms of moist tissue, the phospholipid content is found to be low at birth, to increase rapidly during the first three weeks (123, 192, 263), and then to decrease again. The decrease from the maximum at about the 3rd week is fairly rapid up to the 2nd or 3rd month; as the growth rate slows up, the phospholipid content tends to become constant. The picture is quite different if the weight of phospholipid per 100 grams of dry fat-free tissue is calculated. In this case, the phospholipid fatty acid content is seen to be at the maximum of about 7 per cent at birth and to decrease to about 3.5 per cent during the first 3 months. The rapid increase in the phospholipid content of the moist tissue during the first 3 weeks is thus seen to be a result of the decrease in water content (263).

To what extent the decrease in the phospholipid content of the entire animal during the first few months is due to a change in the relative weights of tissues of differing phospholipid content, and to what extent it is due to an actual decrease in the amount of phospholipid in individual organs, is not yet known. Both probably contribute (263). Thus, the lipid P content of gastrocnemius muscle of rats has been found to decrease by 40 per cent (from 0.09 to 0.05 per cent) between the 3rd and 12th week (61) and a comparable decrease has been found in the phospholipid fatty acids of the carcass( 263). With increase in age the phospholipid content of bone marrow decreases (34, 55, 95a), presumably because of the replacement of the red marrow by the yellow fat marrow.

During post-natal development the phospholipid content of other organs, notably the brain, undergoes a pronounced increase, while in still others no significant change occurs. As shown by Koch and Koch (144) the rapid outgrowth of axons from the nerve cells which occurs in the brain of the rat between the 10th and 20th day after birth is accompanied by a progressive increase in the phospholipid content of the total solids from 12.3 per cent to 21.4 per cent. Likewise, in humans (270, 273) and in dogs (187) the phospholipid content of the brain has been found to increase as development proceeds. It will be noted, however, that the change is due to a change in the histological structure. Indeed there is, as yet, no proof that with increase in age there is a definite alteration in the amount of phospholipid contained in the individual tissue cells, although it seems likely that such occurs in skeletal muscle. On the other hand, it seems equally likely that no change occurs in the liver since the same amount of phospholipid was found in the livers of pups as in the livers of the mothers (300).

In the case of the blood, unmistakable changes in the phospholipid content occur following birth. Curiously enough, the direction of the change differs according to the species of the animal. Thus, the serum of infants (96, 111, 179) and of calves (198) has been found to contain only about one-half as much phospholipid as does adult blood. The same difference is found between the serum of the cord blood of newborn infants and the serum of the mothers (96, 103). The reverse is true of the rabbit, fetal blood being richer in both phospholipid and cholesterol than maternal blood (12).

4. *Effect of hormones.* In view of the effect of various hormones, notably thyroxin, epinephrine and insulin, on the intensity of metabolism and on the nature of the fuel burned, one might reasonably expect that these or other hormones would have a significant influence on the distribution of phospholipids in the body *if* the latter are involved as intermediary products in fat metabolism.

Proceeding on these premises, Artom (4, 5) investigated the effect of removal of the thyroid and ovaries—lack of which reduces basal metabolism—on the phospholipid content of the liver. He found a definite reduction, amounting in ovariectomized rabbits to 22 per cent of the normal average. The average amount of phospholipid fatty acids in 6 normal rabbits was 2.77 grams per 100 grams of fresh liver; in the 5 ovariectomized animals the value was 2.15 per cent. Differences in water or total fat content were not responsible. Artom interpreted these findings as evidence of the intermediary rôle of the phospholipids

in fat metabolism. Thyroidectomy, in addition to the effect on liver phospholipid, has also been found to cause a decrease in the phospholipid content of heart muscle (85, 86); on the other hand, the level in the blood is increased (13, 245). A single subcutaneous injection of thyroid emulsion decreased the blood phospholipid; repeated injections resulted in an increase (245).

Insulin causes a decrease in plasma phospholipid. In rabbits, bled at the onset of convulsions, the values were 35 per cent below the initial value (224). In the dog—especially if the dog is under amytal anesthesia—the decrease in phospholipid roughly paralleled that of the blood sugar, reaching a maximum of 70 per cent of the initial value at about the 3rd hour after injection of the insulin (202). Whether or not insulin has any effect on the phospholipid content of the tissues is still an unsettled question. Theis (295), some years ago, reported that the repeated administration of insulin over a period of 3 weeks and also the single injection of a convulsant dose resulted in a pronounced diminution in the phospholipid content of the livers of rabbits. The average values calculated from Theis' data are as follows: normals, 1.52 per cent; injected with 3 units of insulin per day for 21 days, 0.19 per cent; killed during convulsions caused by 3 units of insulin, 0.99 per cent; killed 6 hours after injection of 3 units, 1.39; injected with 8 units of insulin for 9 days, 1.22 per cent. These findings have not been confirmed. According to more recent work (224) the various organs of rabbits killed at the onset of insulin convulsions contain quite normal amounts of phospholipid. This is evident from the following average values for normal and insulin-treated rabbits, respectively: brain, 6.05, 6.34 per cent; liver, 2.90, 2.98; kidneys, 2.66, 2.53; heart 2.07, 2.09 per cent. In the rats, also, insulin has been found to cause no change in the phospholipid content of the liver (172). Absence of insulin as in pancreatectomized animals causes an increase in the phospholipid content of the liver and a great increase in the fat content (3). The interpretation of this increase is that it is the result of the increased metabolism of fat.

Contrary to what one would expect, the effect of epinephrine on plasma phospholipid is not always, as it is on blood sugar, the reverse of effect of insulin. In fact, the subcutaneous injection of 0.3 to 0.4 mgm. of epinephrine every 15 minutes for 4 hours caused a 28 per cent decrease in the level of phospholipid in the plasma of rabbits (225); that is, about the same decrease as occurred following the administration of a convulsant dose of insulin. In normal dogs, epinephrine has a very

irregular effect on plasma phospholipid. However, if the dogs are under amytal anesthesia, administration of epinephrine resulted in a consistent increase, amounting to about 60 per cent of the initial value (202).

In the same rabbits used for the study of the blood phospholipid, Page, Pasternak and Burt (225) obtained some evidence that epinephrine may cause a decrease in the amount of phospholipid in the brain, kidneys and heart and an increase in that of the liver. However, further work is needed. Fieschi (85) observed no change in the phospholipid content of the heart after injection of epinephine.

Pituitrin, which has repeatedly been shown to cause an accumulation of fat in the liver of the rabbit (62, 63, 316) and, to a less extent, in the liver of the rat (120), has also been found to cause a decrease in the amount of phospholipid in the blood of rabbits (18) and of dogs (18, 230a). George (95), however, was unable to find any change in the phospholipid content of the blood of dogs injected with pituitrin.

Quite recently (255) the claim has been made that there are three distinct substances in the adrenal gland which can be separated by use of various solvents and can be purified by distillation in high vacuum; upon injection into rabbits, one fraction causes a decrease in blood cholesterol, another causes an increase in phospholipid and no change in cholesterol, while the third causes a decrease in phospholipid and no change in cholesterol. What this means remains to be seen.

The changes which take place in the level of phospholipid in the blood as a result of pregnancy and lactation may be discussed at this time, even though it has not yet been shown that they are due to hormone action. In contrast to cholesterol, the phospholipid content of whole blood (219) and of serum (132) is not significantly influenced by menstruation or by ovarian disfunction. But, with the onset of pregnancy, the level of all of the blood lipids including the phospholipids tends to increase, so that pregnant women at term have, as a rule, a higher blood phospholipid value than non-pregnant women (111, 304). Curiously, however, this lipemia of pregnancy which occurs in humans is not observed in dogs (13), while the exact reverse, a decrease, occurs in rabbits (13). The decrease in the rabbit is very striking. In one animal, for instance, the value for lipid P fell from 10.4 mgm. per cent to 4.0 mgm. per cent, the lowest value being reached just before parturition. It may be recalled that the same difference between humans and rabbits was observed in the comparative lipid content of fetal and maternal bloods, fetal blood being lower in lipid than maternal blood in humans and higher in rabbits. One might be inclined to attach some significance

to this correlation were it not for the fact that the cow, like the rabbit, shows a decrease in blood lipids as pregnancy progresses and yet the blood of new-born calves has a low lipid content.

With the onset of lactation in the cow, the level of phospholipid, cholesterol and fat in the blood plasma undergoes a very pronounced increase (197, 198). From a value of about 75 mgm. per 100 cc. of plasma, the phospholipid fatty acids increase to about 175 mgm., the peak being reached in about 1 to 3 months (197). There is a general parallelism in the rise in all the lipids of the blood. It may be assumed therefore that the mammary gland converts the lipids of the blood into milk fat, the increase in the supply being the natural response to the increased demand. The question now arises: Is the phospholipid of the blood plasma the exclusive precursor of milk fat or are the other fatty acid compounds, the neutral fat and cholesterol esters, also taken up by the mammary gland and made over into milk fat? Some years ago Meigs, Blatherwick and Cary (198) made a study of this question and came to the conclusion that under normal conditions the phospholipids alone passed from the blood into the cells of the mammary gland and they alone were precursors of milk fat. The evidence that the mammary gland takes up phospholipid from the blood was obtained as follows: Blood samples were taken from the mammary vein and the jugular vein of lactating and non-lactating cows, the jugular blood being regarded as a fair approximation to the probable composition of arterial blood. The plasmas, and in some cases the whole bloods, were analyzed for total P, inorganic P and lipid P. In the lactating cows, the mammary plasma was found to contain an appreciably smaller amount of lipid P than did the jugular plasma; in non-lactating cows, no difference in the lipid P content of the two blood samples was found. In both lactating and non-lactating cows, the mammary plasma contained more inorganic P than the jugular plasma. This addition of inorganic P to the blood by the mammary glands of non-lactating cows was interpreted as being due to stored phosphate given up during the unavoidable disturbance of the gland caused by removal of the blood sample. In the lactating animals, however, part of the added inorganic P of the mammary blood was believed to have come from the phospholipids which had been taken up and converted into fat. Since milk contains 50 parts of fat to 1 of phosphorus whereas the phospholipids contain 18 parts of fatty acid to 1 of P, the return of phosphate to the blood was to be expected. As the calculated uptake of lipid P was sufficient to account for the amount of fat secreted in the milk, the authors concluded that blood phospholipid was the sole precursor of milk fat.

Simple and attractive though this theory is, it now appears that the question of the origin of milk fat must be regarded as unsettled. According to recent work (17), the use of jugular blood by Meigs and his co-workers as an index to the composition of arterial blood was not a wise one. Actual comparison of blood from the mammary vein with arterial blood has shown that the inorganic P contents are the same. Furthermore, the plasma of lactating cows has been found to contain a significant amount of organic acid-soluble P which appears to be the source of the phosphorus in milk. Since Meigs, Blatherwick and Cary used alcohol-ether mixture as the solvent for their "lipid" P, and since such a solvent is known to dissolve other P-containing substances than the true phospholipids (8, 154), the possibility cannot be dismissed that the "lipid" P which disappeared in the experiments just quoted was not phospholipid P at all.

PHYSIOLOGICAL ACTIVITY. It will be remembered that Mayer and Schaeffer (191) were unable to detect any significant effect of fasting, or overfeeding, or of the type of diet fed on the amount of phospholipid in the various tissues of the animal body. From this "constancy" together with the fact that they are so universally distributed, it was concluded that the phospholipids must have some more general and fundamental purpose than as mere fuel, or, as the authors put it (195) "an important rôle in physiological activity." It ought then to be possible, they reasoned, to demonstrate an appreciable change in the phospholipid content of an organ in response to a sudden demand for increased activity. Accordingly, rabbits and dogs were immersed in cold baths (9°C.) in order to tax to the limit the heat regulating mechanism of the body. In the case of the rabbit, the capacity for increased heat production was quite limited, and the body temperature promptly fell; when it reached 28°C. the animal was removed from the bath. In a few cases body temperature was not restored and the animals died after a few hours; in such animals the liver phospholipid was found to be normal in amount. In most of the rabbits, the body temperature commenced to rise soon after removal from the bath. These were sacrificed at various intervals. In those killed within 2 hours after the temperature had begun to rise, the lipid P content of the liver was found to be definitely below the normal level. Soon, however, the supply became greater than the demand, and between the 3rd and 6th hours the lipid P in the liver rose to a level some 40 per cent above the normal average. After 20 to 24 hours normal values again were found. The only other organs which showed any change were the lungs, in which increases of as high as 100

per cent were found. The dogs, as a rule, were better able to cope with the suddenly increased heat loss. Some suffered no drop in body temperature at all, and in these no change in the lipid P content of the liver was observed. If a drop in temperature did occur, the recovery was very rapid—within 15 to 30 minutes after removal from the bath. The livers of these animals contained a larger amount of lipid P, the maximum being 0.194 gram per 100 grams of dry liver as compared with the normal average of 0.142 per cent. No other organ than the liver was affected.

Recently, Cahn (51) has observed a similar increase in the phospholipid content of the liver during the fever caused by the injection of 1, 2, 4,-dinitrophenol. In 7 experiments the increase in lipid P ranged between 4 and 41 per cent of the amount found in a control sample of the liver removed before injection of the drug.

Here then is evidence that the phospholipid content of the liver is increased by suddenly stimulating the heat production of the animal. What is its meaning? Cahn (51) favors the view that the phospholipids are directly involved in fat metabolism which, of course, is increased markedly. Mayer and Schaeffer (195), on the other hand, are inclined to regard the increase in phospholipid as being a response to the increased demand for oxygen transport. They point out (196) that the increased phospholipid content of the liver is accompanied by characteristic changes in both the number and appearance of the mitochondria in the cells. Although the precise function of the latter is still unknown, there seems to be no reason to believe them to be simple aggregations of phospholipid destined for combustion; on the contrary, they appear to be involved in some more general activity of the cell than that of fat metabolism (65).

Caminade, Mayer and Vallée (53) have made a new attempt to demonstrate the relationship between the intensity of physiological activity and the phospholipid content of the organ. They chose the submaxillary gland. The gland on one side was removed as a control; the other was stimulated to secrete and then analyzed after a period of intense work. Five dogs were injected intravenously with calcium butyrate which causes central stimulation of salivary secretion. The average lipid P content, based on the dry weight of gland, was 0.34 per cent for the resting gland and 0.42 per cent for the secreting gland. A similar change was observed in dogs subjected to reflex stimulation of salivary secretion. Clearly, the production of secretion has been accompanied by an increased phospholipid content of the gland.

These results, however, raise the very important question of how much significance should be attached to a percentage change alone, unaccompanied by a change in the absolute amount present. The secreting gland in the above experiments always weighed less than the resting gland, the difference presumably being the weight of the total solids secreted in the saliva. If the two are assumed to have been of the same weight initially, then a calculation of the absolute amount of lipid P in the resting and secreting glands of the same dog shows that there is no significant difference between them.

The idea that a relationship exists between the physiological activity of a tissue and its phospholipid content has also been put forward by Bloor. As we have seen, the percentage amount of phospholipid differs not only from one organ to another, but from one type of muscle to another. The high phospholipid content of the heart and jaw muscle as compared with the "round" muscle of beef, Bloor (26) attributed to the comparatively greater and more sustained activity of the former. Similarly the high phospholipid content of brain and liver as compared with most other organs was interpreted as an indication of their greater activity. As he has expressed it (29) "the phospholipid content of a tissue (is) an expression or measure of the extent and variety of the physiological activities of that tissue. . . . The term physiological activity as used here is thus meant to include all the processes of the living cell."

In order to demonstrate the relationship between the phospholipid content and the intensity of the activity in a *single* organ, Bloor, Okey, and Corner (29) made an extensive study of the lipid composition of the corpus luteum of the sow at various stages in its progressive development and, in the absence of impregnation, its subsequent degeneration. Others (84, 113, 132) had already noted that the corpora lutea have a high phospholipid content, especially at the time of its full development, so it seemed not unlikely that a definite parallelism could be established between the activity of the gland and its phospholipid content. Such proved to be the case. For the first day or so following ovulation, the corpus luteum was found to have a phospholipid content of about 1.5 per cent. On the 7th day, when the gland is solid and its cells fully differentiated, the phospholipid content has increased to about 2.5 per cent. Thereafter a very rapid increase occurs, so that on the 11th day the phospholipid content is found to be about 4 per cent. If the animal becomes pregnant, the corpus luteum continues to function and the phospholipid content remains high. If, however, fertilization does not

occur, on about the 14th or 15th day a remarkable change in both the histological structure and lipid composition sets in. The gland shrinks in size and the granulosa cells degenerate; at the same time the phospholipid content begins to decrease rapidly, reaching a value of about 2 per cent on the 17th day. During the period of development of the corpus luteum, the free cholesterol content increased somewhat, but much less than did the phospholipid, so that the ratio of $\frac{\text{phospholipid}}{\text{free cholesterol}}$ increased from about 4 during the first week to about 8 at the end of the second week. However, during the degeneration the percentage amount of free cholesterol and, to a still greater degree, of cholesterol esters increased markedly; this increase in percentage amount is thought to be due simply to the shrinkage of the gland, the absolute amount probably remaining constant or decreasing.

Here then was striking evidence that the progressive development of the corpus luteum to an actively functioning gland is accompanied by a 3-fold increase in its phospholipid content and a 50 per cent increase in its cholesterol content. What this means in concrete terms of the function of the phospholipids is still a mystery but it clearly indicates that, whatever it may be, it is of great importance to the proper functioning of the active gland.

A similar but much less striking change was observed in the phospholipid content of the uterine mucosa during the estrous cycle of the sow (220), the maximum content of about 7 per cent being attained at the 7th day after ovulation and then dropping off to about 4.4 per cent by the time of the next estrus. In the placenta of the human, the amount of phospholipid has been found to decrease as the weight increases so that at term the phospholipid content is only about 40 per cent of the value at the 2nd month (311).

All of these changes in the phospholipid content of organs, except in the case of the liver and blood, are gradual responses to a change in the activity of the organ. Acute changes are rare and difficult to produce. This has been repeatedly shown to be true of muscle. Neither in the cat (66) nor in the rat (46) has it been possible to demonstrate any change whatsoever in the phospholipid content of a skeletal muscle forced to contract repeatedly in situ by artificial stimulation, the comparison being made with the phospholipid content of the resting muscle of the opposite leg. (The same is true of the fat as well (46, 66, 318).) Recently Houget (117) has carried out the same sort of experiment on the dog. In the 5 experiments the differences between the resting

and stimulated muscle ranged between 0 and 4 per cent while the maximum difference between the lipid P contents of two resting muscles taken from opposite legs was 15 per cent. However, from the fact that in 4 experiments the stimulated muscle contained the least phospholipid, even though the difference was not beyond the expected error, it was concluded that the phospholipids of the muscle were actually being burned but were being replaced at about the same rate by diffusion from the blood stream. Cahn (51) held the same view of the slight differences observed between muscles taken before and after the fever caused by dinitrophenol. However, it is obvious that the absence of any significent change in amount of phospholipid in skeletal muscle following a working period can with equal validity be interpreted as evidence that they are not involved as intermediaries in the metabolism of fat.

THE SIGNIFICANCE OF THE PHOSPHOLIPID TO CHOLESTEROL RATIO. The phospholipids, as we have seen, are invariably present in every tissue cell, the amount being constant and yet characteristically different from one organ to another. The same holds true of cholesterol. One finds therefore that the amount of phospholipid in each individual organ bears a certain characteristic relationship to the amount of cholesterol. This relationship is known as the $\frac{\text{phospholipid}}{\text{cholesterol}}$ ratio. Brain though rich in phospholipid is, comparatively speaking, still richer in cholesterol so that it has the lowest ratio of all organs of the body with exception of the adrenal gland. Muscle, though poor in phospholipid, is still poorer in cholesterol, so that it shows the highest ratio. Now, what is the significance of this phospholipid: cholesterol ratio?

It is very generally believed that phospholipid and cholesterol have antagonistic actions within the body—even though very little of a precise nature is known as to the actions of either. There are, however, certain justifications for such a belief.

If both the phospholipid and cholesterol content of a tissue were to remain rigorously constant under all circumstances, no greater significance could be attached to the constancy of the ratio between these two than between either one of them and the protein or water content of the cell. It is in the changes which occur, both physiological and pathological, that the clue is found which suggests their probably antagonistic action. In most instances in which a change occurs in the phospholipid content of a tissue, a change in the same direction, though not always to exactly the same extent, also occurs in the cholesterol content. Thus,

in the liver, both phospholipid and cholesterol increase following pancreatectomy (3) and decrease following ovariectomy (5). The parallel changes in phospholipid and cholesterol are especially noticeable in blood plasma; both fall when the diet is low in fat and rise when it is high in fat (30, 176); both rise with the onset of lactation in the cow (197); during pregnancy both rise in the human (111, 304), and fall in the rabbit (13); both rise in the lipemias occurring in diabetes, nephrosis and following prolonged hemorrhage (see p. 381). It has been known for some time that the feeding of either fat alone or with cholesterol leads to an increase in all three lipid constituents, fat, phospholipid and cholesterol, in the blood stream (30, 119, 201). Likewise injection of cephalin leads to an increase in cholesterol as well as phospholipid in the blood plasma (227).

There are, however, exceptions to this parallelism between phospholipid and cholesterol changes. For instance, the increase in the phospholipid content of the liver (7) and blood plasma (31, 82) during fat absorption is not accompanied by any alteration in the cholesterol value. Likewise profound variations may occur in the level of cholesterol in the blood, such as throughout the menstrual cycle (133, 219), with the phospholipid level remaining practically constant. Probably the most striking example of the possibility of there being an enormous shift in the ratio of phospholipid to total cholesterol without any untoward symptoms at all is that which occurs in the livers of rats which have been fed cholesterol for some time. With normal or even slightly subnormal values for phospholipid, the total cholesterol content may increase as much as 30-fold. However, it must be pointed out that the great increase in total cholesterol is almost entirely in the ester fraction, the values for free cholesterol being practically normal (57, 221). And since most of the cholesterol present in normal tissues is in the free condition (the occurrence of esters being an indication of degenerative processes involving the splitting of phospholipids with the liberation of free fatty acids which then esterify with cholesterol (29)), it is more correct to think only of a balance between *free* cholesterol and phospholipid.

Most of the experimental work which has been done on phospholipid and cholesterol antagonism indicates that it is a function of their physical chemical properties and the theories which have been developed to explain that antagonism conceive of the phospholipids and cholesterol as being primarily important to the maintenance of a certain optimum colloidal state of the cell. This idea that the physiological importance of the phospholipids lies in their hydrophilic properties which, properly

balanced by the hydrophobic properties of cholesterol, influence the water and salt exchange of the organism, has been developed in great detail by Degkwitz and his school (69).

The theory that the ratio of phospholipid to cholesterol played an important part in the water balance of the cell was put forward by Mayer and Schaeffer in 1914 (193, 194). *In vivo*, the water content of the various tissues bore no relationship to their phospholipid and cholesterol contents; but, if the tissues were immersed in water under standard conditions, the imbibition proved to be inversely proportional to the $\frac{\text{phospholipid}}{\text{cholesterol}}$ ratio. In this respect, their findings are directly opposite to those of Degkwitz since, according to the latter, the higher the content of cholesterol, the less would be the uptake of water. Indeed, it was found that the intravenous injection of lecithin emulsions favored the retention of water and of chlorides; injection of cholesterol suspensions, on the other hand increased the output; combination of lecithin with the cholesterol diminished the effect of the latter (67, 68, 69).

The resistance of the red blood cell to the hemolytic action of hypotonic salt solutions and snake venoms is seemingly dependent on the relative proportions of phospholipid and cholesterol in the enveloping membrane. Washing the red cells increases their resistance to hypotonic hemolysis because of the removal of lecithin from the surface of the cell (41); similarly, feeding rabbits on a low fat diet which leads to a reduction in the phospholipid content of the blood, increases resistance to hemolysis. *In vitro*, the addition of lecithin decreases and of a cholesterol suspension increases resistance of the red cell to the hemolytic action of hypotonic salt solutions and of acids and bases (279). It has long been known that beef red blood cells are resistant to the hemolytic action of cobra venom but lose their resistance in the presence of lecithin (148). Hemolysis by venoms, however, is due to the formation of lysolecithin or lysocephalin by the action of an enzyme in the venom, the lyso-phospholipids being the active hemolytic agents (70, 184).

The sedimentation rate of the red cells has also been thought to depend, in part at least, on the relative concentration of phospholipid and cholesterol on the surface. However, while some have been able to obtain evidence in favor of such a view (42, 285), others have been unable to do so (204, 218, 314).

It has been claimed that the antagonism between cholesterol and lecithin can be demonstrated directly by intravenous injection; cholesterol was found to cause anesthesia and, on injection of lecithin, the

animal recovered (54). However, lecithin injections have no effect at all on the induction of, or recovery from, anesthesia caused by ether, chloral or morphine (147) and, though others have injected cholesterol, no mention of its anesthetic properties has been made (67, 69). Similarly the observation that cholesterol inhibits the oxidation of phospholipid *in vitro* (258) appears to lack any confirmation.

It has frequently been claimed that both lecithin and cephalin possess well-marked pharmacological properties. Unfortunately, in many cases insufficient attention has been paid to the purity of the substance used. The importance of working with highly purified preparations can best be emphasized by citing an example. Clark (59) found that lecithin from egg yolk had a restoring action on the frog's heart made hypodynamic by prolonged perfusion. When repeated with the more highly purified egg and brain lecithin prepared by Eggleton (74), it was found that the stimulating property was not associated with the phospholipid but rather with the water-soluble impurities. Similarly, Nielson (217) was unable to detect any appreciable effect of purified lecithin or cephalin on the frog's heart, although others (281), using cephalin prepared and purified by the same method (Levene and Rolf) (157), observed a stimulating action. It may be remarked, however, that the constitution of even highly purified cephalin is still uncertain.

PATHOLOGICAL FACTORS AFFECTING THE PHOSPHOLIPID CONTENT OF TISSUES. By far the most remarkable instance of a pathological disturbance in the phospholipid content of blood and tissues is that observed in the Niemann-Pick type of lipoid histiocytosis (242). This disease occurs only in infancy or in early childhood and is invariably fatal. Grossly, the disease is characterized by enormous enlargement of the liver and spleen; histologically by a proliferation of the reticuloendothelial system; and chemically by abnormally large amounts of phospholipid in the liver and spleen, and, to a lesser degree, in the brain (77, 79, 80, 171, 276). The phospholipid content of the liver may be 10 times and that of the spleen 8 times the normal value (80). The cholesterol content of these organs is also increased somewhat but the 60 per cent increase (78) is small as compared with the phospholipid. The underlying cause of the accumulation of phospholipid is unknown. Sobotka (277) has suggested that there has been a derangement in the normal balance between the various esterifying reactions in which fatty acids are bound up in fat, phospholipids, cerebrosides, and cholesterol esters. Since the blood phospholipid is approximately twice the normal level (171), it seems likely that the disturbance is a general one, and

that the reticulo-endothelial cells in the spleen and liver have taken up their phospholipid from the blood. Niemann-Pick's disease has its counterpart in Gaucher's disease, in which there has been an overproduction of kerasin, one of the cerebrosides, and in the Schüller-Christian disease, in which cholesterol and cholesterol esters accumulate.

Ever since the discovery early in the last century that the brain and nervous system in general are especially rich in fatty substances, efforts have been made to connect up diseases of the nervous system with abnormality in the lipid content or balance. Up to the present these efforts have not been very successful. However, in certain instances abnormality in the lipid content of the brain can be detected. Thus in general paralysis, the total lipid and especially the phospholipid content of the brain is below normal (145, 272); the change is more noticeable in the cerebrum than in the cerebellum or spinal cord, and more pronounced in the cortex than in the corpus callosum. However, even in the cerebrum the abnormality is not great. In paralytics the phospholipids made up 22.7 and 25.3 per cent of the total solids; in normals the values are 28.5 and 28.1 per cent (145). In the case of progressive paralysis, in which histological examination shows lesions in the frontal lobes of the cerebrum and degeneration of the myelin sheaths, an increase, rather than a decrease, in phospholipid has been observed (271). However, the same increase in phospholipid was also found in cachexia unaccompanied by any clinical involvement of the nervous system. Wallerian degeneration of a peripheral nerve brought on by cutting the nerve is accompanied by a decrease in the phospholipid content to about 10 per cent of the normal value (190). It is noteworthy that the disappearance of phospholipid does not set in until the end of the first week; by the end of the 2nd week, about one-half of the initial amount has disappeared.

1. *Lipemias.* Because of the comparative ease with which blood may be obtained for analysis, a very considerable amount of work has been done concerning the effect of disease on amount of phospholipid and other lipids in whole blood or plasma. A list of some of the various diseases which have been observed to cause an increase or a decrease in the level of phospholipid in the blood is given below, with a few leading references.

Diseases in which there is an *increased* blood phospholipid: diabetes (21, 99, 127); nephrosis (159, 167); Niemann-Pick's disease (171); chronic hemorrhage (83, 210); epilepsy (35); hypertension (82); syphilis (215); hypothyroidism (13); liver injury by P and $CHCl_3$ (212); hydrophobia (324); B-avitaminosis (121, 283).

Diseases in which there is a *decreased* blood phospholipid: infectious fevers (181, 188); cancer (71, 189, 234); epilepsy (182, 183); pernicious anemia (20, 209); B-avitaminosis (201).

In some of the investigations the work was well controlled and the changes observed are undoubtedly significant; in others, physiological factors such as diet, which can affect the lipid content of the blood so materially, have not been adequately controlled so that the significance of the findings is uncertain.

Of the diseases which quite definitely are accompanied by abnormality in the level of blood phospholipid, three are outstanding—diabetes, nephrosis and chronic hemorrhage. While very different from a clinical standpoint, these three are similar in that the increase in phospholipid is accompanied by an equal or greater increase in fat and cholesterol. In other words, there is a general lipemia. The degree to which these lipids will accumulate in the blood stream is sometimes quite astonishing. Bloor (21) observed in a case of severe diabetes that the fat was 26 times, the phospholipid 5 times and the cholesterol 9 times, the values for normal humans.

What is the meaning of this enormous increase in blood lipids? It is a common feature of both alimentary and pathological lipemias that the order of increase of the blood lipids is, first the fat, then the phospholipid, and lastly the cholesterol. As a rule, the cholesterol does not rise after a single fat feeding but only after prolonged feeding (21, 30, 31). It follows then that the cause of the increase in the phospholipid, and possibly the cholesterol as well, lies in the increase in fat. A discussion of the reason for the latter need not concern us here. Suffice it to say that several explanations have been offered—impairment in the ability to store fat as in diabetes; flooding of the blood with fat from one of the stores as in hemorrhage; diminished lipolytic activity of the blood; and as a means of compensating for the diminished colloid osmotic pressure of the blood resulting from the depletion of plasma proteins, as in hemorrhage and nephrosis (21, 89, 90, 201). The explanation of the increase in blood phospholipid depends upon the point of view concerning their function. If they are intermediary metabolites involved in the transport of fatty acids for combustion, then it can be readily seen that the increased amount of fat in the blood and liver has led to an increase in the intermediary products even though, as in nephrosis or hemorrhage, there may be no increase in fat metabolism. If, on the other hand, one attributes some other function to the phospholipids then an explanation of their increase is more difficult. It may be that they increase simply

because of their solubility in the blood fat, as has been offered as an explanation for the cholesterol increase (244), or else as a means of stabilizing the emulsion of the blood fat. Whatever the explanation, the increase in blood phospholipid during lipemia in the rabbit is not accompanied by any detectable change in the phospholipid content of the organs (110).

The question of the physical state of the phospholipid in blood plasma comes up at this point. It has long been known that the lipids cannot be extracted from blood plasma by simple treatment with fat solvents such as ether or benzene. If alcohol is added to the ether, then good extraction is obtained. This fact suggests that the lipids are bound in some manner with the blood proteins. Within the past few years, evidence has appeared which shows that such a combination between the protein and lipids of the blood does actually occur. Thus Macheboeuf (165, 166) was able to obtain a protein fraction which contained 59 per cent protein, 23 per cent phospholipid and 18 per cent cholesterol and which did not alter its composition on further resolution and reprecipitation. This lipid-protein complex is soluble in water and with it aqueous solutions containing 5 per cent of lipid can be obtained. Also, many (1, 296, 303) have found that the blood proteins, especially the globulins, carry down with them on precipitation with $(NH_4)_2SO_4$ a considerable portion of the lipids. On the average, the proteins carried down about 50 per cent of the total lipid, 30 per cent of the phospholipid, and 70 per cent of the total cholesterol of the blood plasma (303). Correlated with this is the fact that the phospholipids of the blood and more especially the cholesterol are held back by the capillary wall to about the same extent as the blood proteins, and that there is a rough parallelism between the protein and lipid contents of transudates (186, 170). In lipemic serum, such as in nephrosis, a much smaller proportion of the lipids is bound to the proteins since 70 to 87 per cent can be extracted directly with ether as compared with only 30 per cent from normal serum (169), the amount of lipid-protein complex being practically normal (168).

Although the changes in the blood lipids are most pronounced in diabetes, nephrosis and chronic hemorrhage, the lipid changes in other diseases are probably also of considerable importance. Some emphasis has been placed on the abnormality in the blood lipid levels of cancer patients in whom there is a tendency for the phospholipid to be low and the cholesterol high (71, 189). Recently a causal relationship between epileptic convulsions and the ratio of phospholipid/cholesterol in the

blood plasma has been suggested. In the plasma of epileptic children the cholesterol is normal but the phospholipid is significantly below normal—125 mgm. per cent as compared with 175 mgm. per cent. Diet was not a factor (182). In severe epileptics, there was a correlation between the diurnal variation in the lecithin/cholesterol ratio and the time of onset of convulsions. In most of the cases, the convulsions came in the forenoon and in the forenoon the ratio was higher than in the afternoon. In a limited number in whom convulsions occurred in both the forenoon and afternoon, no decrease in the ratio occurred during the day (183). As a rule, the variation in the ratio was due more to variations in the phospholipid than in the cholesterol. McQuarrie and Stoesser have shown recently that infectious fevers, but not artificial fevers, are accompanied by a drop in both phospholipid and cholesterol in the blood plasma (181). In fact, artificial fevers may cause an increase in both the phospholipid and total fatty acid content of the plasma (51).

2. *Avitaminosis.* A deficiency of vitamin B appears to have a very real effect on the lipid content of the blood and organs. Rats (283), rabbits (121), pigeons (201, 250) all develop a lipemia as the disease progresses. The lipemia of the pigeon is rather unique in that the phospholipid goes down as the fat and cholesterol increase. The same change occurs in the organs of the pigeon. All show a pronounced increase in the cholesterol content but the phospholipid content decreases by 18 to 40 per cent in brain, liver, kidneys and lungs, while skin and muscles show no change (201). Schmitz and Hiroaka (251) interpreted the fall in phospholipid during vitamin B deficiency to be an indication of an impairment in the rate of synthesis and accordingly administered lecithin, both by mouth or parenterally, to pigeons on the deficient diet in the hope of alleviating at least part of the symptoms. While the life of the birds was prolonged somewhat, the ultimate outcome was the same and the fall in blood phospholipid was not prevented. Later the experiment was repeated with brain cephalin but no beneficial results at all were observed (253).

There is still some reason to doubt that the effects of vitamin B deficiency are in any way specific to that single deficiency since in rats deprived of either vitamin B or vitamin A the liver phospholipid was observed to be only $\frac{1}{2}$ to $\frac{1}{3}$ the normal (205). Also the gastrocnemius muscles of young rats deprived of vitamin D are poor in acid-insoluble P (i.e., chiefly lipid P) (61), presumably because of the abnormal phosphorus metabolism.

3. *Poisons.* Although the fat content of the liver is known to be increased enormously by administration of chloroform or phosphorus, there is still some doubt as to their effect on the phospholipid content. Some (109, 295) have claimed that phosphorus poisoning causes a decrease in liver phospholipid; others (102, 162, 241) have claimed an increase and over against these we can put the findings of others still that neither phosphorus, nor diphtheria toxin, nor a trypanosome infection (249) nor chloroform (172) caused any change whatsoever. The phosphorus and trypanosome infection did, however, cause an increase in the fat content of the liver. Reduced atmospheric pressure, which has been shown to cause a slight drop in the phospholipid content of blood plasma (282) and which causes a marked increase in the fat content of the liver, has been claimed (102, 162, 206) to cause an increase in the liver phospholipid as well. However, the methods used—direct ether extraction of dried liver—were so unreliable that no conclusion can be drawn. Repeated injection of alcohol over a period of several months was found to decrease the phospholipid content of frogs to about 60 per cent of the value for normal frogs injected with saline (150).

4. *Tumors.* The recent enormous increase in the interest in the growth and metabolism of tumors has, as might be expected, resulted in an extensive study of their lipid metabolism. One extremely important fact, which seems to be well substantiated, is the greater amount of both phospholipid and cholesterol in malignant than in benign tumors (14, 130, 320). What this means is not at all clear. Since there is some evidence that the tissues of young rapidly growing animals have a higher phospholipid content than older animals (see p. 367), it may be that the rapid growth of malignant tumors is related to the high phospholipid content. However, the phospholipid content of tumors is scarcely as high as that of parenchymatous organs such as the liver and kidney (45). Quite a bit of emphasis is being placed on the ratio of the phospholipid to cholesterol in tumors, though various authors differ in their opinions as to the relative functions of the phospholipids and the cholesterol. Some attribute the rapid growth to the high cholesterol content (239, 293), the phospholipids being growth-retarding substances; according to others (14, 130, 156) the rôles are just reversed. In a single strain of experimental tumors (Jensen sarcoma), the rate of growth decreases as the phospholipid/cholesterol ester ratio decreases (15); the phospholipid content (and free cholesterol) is relatively constant, the decrease in the ratio being due almost entirely to the increase in the cholesterol esters. As the tumor ages, the phospholipid content decreases while the free

and combined cholesterol increase (305). Pronounced differences are found in the lipid content of the outer growing portion of the tumor and the central necrotic portion; the former is rich in phospholipid and free cholesterol but relatively poor in cholesterol esters, while the latter is poor in phospholipid and rich in both free and combined cholesterol. Presumably the necrosis is accompanied by hydrolysis of the phospholipid, part of the liberated fatty acid being bound up with cholesterol (15). Others, on the other hand, have found a higher proportion of cholesterol to lecithin in the periphery than in the center of the tumor (40). Since differences occur among the various types of tumors (15), great care must be exercised in making generalizations from observations on a single type.

THE COMPOSITION OF THE PHOSPHOLIPIDS. Thus far the discussion has been limited to the available information concerning the *amount* of phospholipid in animal tissues and the significance of such changes as occur under various physiological and pathological conditions. There remains still another feature of the phospholipids, quite as important as their amount, namely, their *composition.*

There are two angles from which the composition of the phospholipids must be considered: first, from the point of view of the relative proportions of the three members, the lecithins, cephalins and sphingomyelins: second, from the point of view of the relative proportions of the various individual lecithins and cephalins which, as has been pointed out, differ from one another only in the nature of the two fatty acids in the molecule.

It is unfortunate but nevertheless true that next to nothing is known at present concerning the relative importance of the lecithins, cephalins, and sphingomyelins. From the fact that the lecithins and cephalins make up the bulk of the phospholipids in most organs one can safely infer that they fulfill such functions as we are able to attribute to the phospholipids as a whole. But which of these functions are taken over by the lecithins and which by the cephalins is almost a complete mystery. As for the sphingomyelins we know only that they occur in brain and nervous tissue, the adrenals, kidneys, liver, spleen, heart, and red blood corpuscles (298). The lipids of the stromata of the red blood corpuscles have, indeed, been found to consist of sphingomyelin, cephalin, and cholesterol in approximately equal amounts (47). Since the stroma of the red cell is essentially a membrane structure (presumably much like a sponge), one sees here a hint of the possibility that the sphingomyelin is an important component of the much-discussed lipid membrane

of all cells (94); if the latter does exist at all, it probably contains but a small portion of the total phospholipid of most tissues. This conclusion was arrived at by calculating the approximate total area of the cells (in the liver, for example) and comparing the amount of phospholipid necessary to give a mono- or di-molecular layer—using the data of Adam (2)—with the amount actually present. The absence of lecithins from the stroma of the red cells of humans (47), sheep (47) and horses (107) was established by the failure to detect any choline in the ether-soluble phospholipids and also by the fact that all of the nitrogen was in the amino form. In view of the high sphingomyelin content of the red cells, it appears not unlikely that the lignoceryl-sphingosine which has been found in liver and spleen (93, 294, 301a) has its origin in the damaged red cells which are taken up in these two organs.

One well-established function of the cephalins is their rôle[2] in the process of blood coagulation. The lecithins are completely inactive (98, 118, 180, 309). According to the recent findings of Fischer (88), which confirm the views held by Bordet (36) and others (203), the cephalins combine with a protein and calcium to form thrombin which is known to be the factor responsible for the conversion of fibrinogen into fibrin. Others (309) believe that the formation of a true calcium salt of cephalin is an essential step in the process of blood coagulation.

Recently Schmitz and Koch (252) have developed a micro method for the determination of cephalin and lecithin in amounts of 2 to 10 mgm. which should prove exceedingly useful in determining the relative proportions of these two phospholipids in various tissues. Up to the present they have worked only on blood plasma. They found that in most animals the cephalins make up about 25 to 30 per cent of the total phospholipid of the plasma. In the normal rabbit, however, the cephalin makes up about 90 per cent of the whole, but when the phospholipid is increased, as in hemorrhagic lipemia, then the ratio of cephalin/lecithin decreases. The increase in the percentage amount of cephalin in the plasma of the rabbit following repeated hemorrhage has been held to be responsible for the decreased clotting time (252). It is known that the liberation of cephalin from the blood platelets is responsible for the clotting of shed blood, and since the circulating blood contains cephalin, it appears not unlikely that it is bound to the blood proteins and therefore inactive.

[2] The uncertainty concerning the composition of cephalin, including that used in studies of blood coagulation, always leaves open the possibility that its thromboplastic activity is due either to some as yet unrecognized constituent of the molecule or to some firmly associated contaminating substance.

Concerning the second way in which the composition of the phospholipids may differ from organ to organ and from time to time, namely, in the nature of the fatty acids contained in the molecules, a fair amount of information is at hand. Each molecule of lecithin and cephalin contains two fatty acids. Now, since seven fatty acids—palmitic, stearic, oleic, linoleic,[3] gadoleic, arachidonic, and clupanodonic—have been satisfactorily identified (298) as constituents of the phospholipids (exclusive of the sphingomyelins), and others still unidentified probably occur, it follows that several different lecithins and cephalins are present in animal tissues. Just how many different combinations of these fatty acids do occur is still unknown, but a large number is theoretically possible, especially since both $\alpha$- and $\beta$-forms occur (321).

It will be observed that palmitic and stearic are the only two saturated acids found in the lecithins and cephalins, while at least five and probably more unsaturated acids are present. For some time it has been believed that each molecule of lecithin and cephalin must contain one saturated and one unsaturated fatty acid (158). There is now good reason for believing that that is not true. Thus, a lecithin containing two molecules of palmitic acid has been detected in brain (199), and the same organ has been found to contain lecithins in which both fatty acids are unsaturated (321). Furthermore, several (138, 267, 275) have found that the saturated acids amount to only 30 or 40 per cent of the total so that, even allowing for higher mean molecular weight of the unsaturated acids, a considerable portion of the phospholipid molecules must contain two unsaturated acids. Because of this preponderance of unsaturated acids and especially because of the presence of the highly unsaturated arachidonic and clupanodonic acids, the fatty acids contained in the phospholipids of animal tissues are, in general, characterized by a high degree of unsaturation in comparison with the fatty acids bound as triglycerides. It is only natural therefore that the function of the phospholipids in the body has been regarded as being connected in some way with their high degree of unsaturation.

According to Leathes (151, 152), desaturation of the fatty acids is a primary step toward their ultimate combustion in the tissues. The finding of practically all of the highly unsaturated acids of the body in combination as phospholipids rather than as neutral fat can therefore—if the Leathes theory is correct—scarcely be interpreted otherwise than that the phospholipids in some way facilitate the transport of the readily oxidizable fatty acids or, in the original sense of Loew, actually serve as

[3] Linolenic acid, the three-double-bond $C_{18}$ acid, appears to occur only in traces, if at all (137, 275).

a machine for burning them. An alternative explanation of the high degree of unsaturation of the phospholipids is that the double bonds readily take up oxygen, not for the purpose of burning the fatty acids *per se*, but rather as a means of conveying it to some oxidizable substance within the cell. According to the third alternative no special significance is attached to unsaturation save in so far as it modifies the physicochemical properties of the phospholipid molecule. That the presence of one or more unsaturated linkages in the long chain of carbon atoms of the fatty acids does have a profound effect on such properties as are involved in molecular orientation on surfaces (2) and in surface and interfacial tension, can not be doubted. And it may well be that in such a way a high degree of unsaturation of the phospholipids works to the advantage of the living organism.

In any case it is evident that the degree of unsaturation—which, it must be remembered, reflects the relative proportions of lecithins and cephalins of different compositions—, is probably of prime importance to their function, whatever it may be. And it is equally evident that if we can learn something about the factors which govern the degree of unsaturation of tissue phospholipids we ought to find some clue as to their function.

At present the only factors which are definitely known to have a decisive influence on the composition of the phospholipids are the nature and amount of fat in the diet. As long ago as 1910 Joannovics and Pick (125) demonstrated that the iodine number of the phospholipid fatty acids of the liver could be made to increase from about 100 to 150 within the space of a few hours by feeding cod liver oil, a fat of high I. N. Being especially interested in the liver they did not extend their studies to other organs. The same was true of Shioji (259) who showed that the phospholipids in the liver had a different degree of unsaturation in rabbits fed coconut oil than in those fed linseed oil (also 146). The liver, however, is not the only organ affected. In all organs thus far studied—brain, heart, kidneys, liver, intestinal mucosa, intestinal muscle, and skeletal muscle (261)—and in the blood (269), it has been possible to show that the composition of the phospholipids depends upon the diet of the animal. Brain has been included in the above since, although it is not yet definitely proven, there is no reason for believing that it is an exception to the rule. The composition—but not the amount (288)—of the phospholipid in the hen's egg (112, 178, 287) and of rat fetuses (262) is also affected by the diet.

This relationship between food fat and the tissue phospholipids has

been extensively studied in the white rat because of the ease with which it can be raised on the so-called synthetic diets. When rats are raised on a diet which is practically free of fat, the phospholipid fatty acids of the tissues are found to have a certain characteristic low iodine number. The addition of any of the common fats causes an increase above this minimal level. The size of the increase, however, is not, as one might expect, proportional to the iodine number of the fat fed, but depends rather on the particular types of fatty acids present. Thus, olive oil and linseed oil have about the same effect, while cod liver oil, presumably because of the highly unsaturated long-chain fatty acids which it contains, has a very much greater effect (264). The saturated acids of the food fat apparently do not have any effect at all on the composition of the phospholipids since feeding large amounts of hydrogenated coconut oil caused neither an increase nor a decrease from the level characteristic of the fat-poor diet. Also it has been found that a small amount of unsaturated fat has just the same effect, regardless of whether it is added to the fat-poor base or to a diet containing a large amount of saturated fat (269). One may conclude therefore that under normal living conditions, the rat, when synthesizing phospholipids, makes use of the unsaturated fatty acids which it takes in with its food in preference to those of lower iodine number which it synthesizes from carbohydrate. At the same time it always uses some saturated acids in building up its phospholipid since, regardless of the type of diet and therefore regardless of the average degree of unsaturation, the total phospholipid fatty acids have been found to be made up of 30 per cent saturated and 70 per cent unsaturated acids (267). Why there should be this fixity in the ratio of saturated to unsaturated acids and yet a great variability in the make-up of the latter is still unknown.

As is to be expected, the amount of fat in the diet is just as important as its composition. By supplementing the fat-poor ration with increasing amounts of a single fat it was found that a definite quantitative relationship existed, the iodine number of the phospholipid fatty acids being directly proportional to the logarithm of the amount of fat fed. However, this was true only of the entire animal and the skeletal muscle, not of the liver (265). Because of the logarithmic nature of the relationship, very small amounts of unsaturated fat in the diet have a great effect, a fact which may explain the failure of Terroine and his school (287, 290) to find that diet fat had an influence on the composition of the phospholipids other than those of the egg.

The amount and nature of the food fat are not, however, the only fac-

tors governing the degree of unsaturation of tissue phospholipids, though what the other factors are is unknown. There is evidently some factor responsible for the selection of certain fatty acids and the rejection of others, and for the maintenance of a fixed ratio of saturated to unsaturated acids. Also there appear to be consistent and significant differences from one organ to another in the same animal (261, 291). Environmental temperature has been shown to exert a very considerable influence on the degree of unsaturation of the phospholipids of moulds and bacteria (291), as well as that of the total fatty acids (228, 289, 291, 323). An increase in the intensity of metabolism by administering thyroxin has also been shown to have a bearing on the unsaturation of depot fat (231) but whether or not the same applies to the phospholipids has not yet been shown.

The pronounced effect of small amounts of food fat opens up the very important question of the synthesis of unsaturated fatty acids by the rat. The recent studies of Burr and his co-workers (48, 49, 50) and of others (81) have proven very clearly that for the normal growth and well-being of the rat, the presence of either linoleic or linolenic acid in the diet is essential. Neither oleic acid nor saturated acids can replace them. These facts led Burr to the conclusion that the rat and other warmblooded animals are unable to form linoleic or linolenic acid either by synthesis from carbohydrate or by desaturation of oleic or stearic acids. Now, the iodine numbers of the phospholipid fatty acids in the tissues of rats raised on "fat-free" or fat-poor diets, though unusually low, are still so high—never below 112 in the liver and 81 in carcass—that it is impossible to avoid the conclusion that acids more highly unsaturated than oleic are present. And when allowance is made for the 30 per cent of saturated acids, the amount of these more highly unsaturated acids becomes appreciable. The question is: What is the origin of these unsaturated acids? Were they obtained from the "fat-free" or fat-poor diet? Were they present in the tissues and fat stores of the animal when placed on the diet? Or were they synthesized? The first possibility is obviously strengthened by the proof of the pronounced effect of small amounts of unsaturated fat in the food (265); the second is supported by the demonstration that highly unsaturated acids are retained with great tenacity within the phospholipid molecule (266, 268). There is, however, one important fact which makes it impossible to dismiss entirely the possibility that these highly unsaturated acids were actually synthesized by the rat. That is the occurrence of large amounts of arachidonic and clupanodonic acids in the tissue phospho-

lipids and liver fats of the exclusively herbivorous animals such as the cow (43, 44, 138, 275), pig (43) and sheep (43). Recently they have been found present in the livers of frogs (140). Since, so far as is known at present, plants do not contain these highly unsaturated acids, one cannot avoid the conclusion that they must be synthesized by the animal.

There is no doubt however that, on the fat-poor diet, the rat's supply of unsaturated fatty acids is limited and that the presence of unsaturated acids in the diet improves the growth rate and increases the degree of unsaturation of the newly synthesized phospholipid. To what extent the two are causally related is still unknown, but one is tempted to assume that the degree of unsaturation of the phospholipids of the rats on the fat-free diets is too low and their functioning is therefore impaired. However, it may be pointed out that oxidation in the tissues of such rats is above rather than below normal as one might expect if the phospholipids are engaged in oxygen transport (313). In view of the ineffectiveness of the oleic acid as a growth-promoting agent, it would be of great interest to know what effect it would have on the phospholipids. Since olive oil and linseed oil have about the same effect, it may be that only the linoleic and linolenic acid in the former are effective. Incidentally it may be pointed out that linolenic acid, which is quite effective as a growth-promoter, is not present in detectable amounts in the phospholipids (137, 275).

Now, what is the bearing of all these facts on the question of the function of the phospholipids? At first glance one might say that the influence of food fat on the degree of unsaturation of the phospholipids is just what one would expect if the ingested fatty acids are built into phospholipid and conveyed to the tissues to be burned. On the other hand, if the phospholipids are essential tissue constituents which take part in oxygen transport or in some other process in the cell, it may be assumed that they are subject to wear and tear and that the unsaturated fatty acids taken in with the food are used in the repair process. The essential difference between these two interpretations lies in the implied rate of turnover of phospholipids. If they are intermediary metabolites, the rate of turnover must be rapid; if due to wear and tear only, then turnover should be relatively slow.

Having established the fact that the degree of unsaturation of the phospholipids in the tissues of the rat is characteristic of the type of diet fed, Sinclair (266) adopted as a measure of the rate of turnover the rate at which the iodine number increased or decreased from one level to another following a change in the diet. By this means it was found that

when the rats were changed from their customary fat-poor diet to one containing cod liver oil, the turnover of liver phospholipid was complete in 2 or 3 days; the turnover of muscle phospholipid however was much slower, being 50 per cent complete in 3 days and only about 80 per cent complete in two weeks. Contrary to what was to be expected if the phospholipids are intermediary metabolites, the rate of turnover was found to be unaffected by changing the intensity of fat metabolism. The most striking finding, however, was that the rates of increase and decrease in iodine number were very unequal. When the rats were changed over to a diet which, because of a low content of highly unsaturated acids, leads to a low degree of unsaturation, the iodine number of the phospholipids fell very slowly. Evidently the tissue phospholipids have an interest, so to speak, in conserving their highly unsaturated acids when the supply in the food is cut off. These facts, together with others—such as the failure of saturated acids to have any effect on the phospholipids, the pronounced effect of minute amounts of unsaturated fatty acids, and the predominant influence of the highly unsaturated long chain fatty acids of cod liver and menhaden oils—all lead to the conclusion that the purpose of the high degree of unsaturation of the phospholipids is not to make them more easily burned but rather to enable them to fulfill some function in the cell other than that of a simple fuel. What that function is, whether it is to act as an oxygen transfer agent, or to take part in a strictly physical way in the activity of the cell, must be left for the future to decide.

In so far as muscle is concerned the conclusion that its phospholipids are not intermediary products in fat metabolism is in agreement, for the most part, with the failure to observe significant changes in the amount during greatly increased activity. But the same is not true of the liver, nor is it true of the blood. In these two organs very appreciable changes in phospholipid content occur when there is an increased transport or catabolism of fat—such as during fat absorption, during fasting, and during increased heat production. These changes fit in well with the view that the liver plays a very special rôle in fat metabolism, being responsible both for desaturation of the fatty acids and for building them up into phospholipids for transport by the blood to the tissues where they are burned. However, it may be pointed out that the phospholipids of the blood plasma do not have as high an iodine number as would be expected; the highly unsaturated acids of the blood appear to be bound up as cholesterol esters rather than in the phospholipids (23, 24, 56, 248). Recently direct evidence has been brought forth to show that there is an

actual decrease in the phospholipid content of the isolated perfused liver if fat is being burned but not if carbohydrate is being burned (128). Furthermore, it was found that addition of cephalin to the blood perfusing a normal glycogen-containing liver caused a prompt increase in $O_2$ consumption, a fall in R. Q. and an increased acetone body production; when the same experiment was performed on the fatty livers of phlorhizinized dogs, again there was an increase in $O_2$ consumption and a fall in R. Q. but, instead of an increased acetone body production, there was an increase in sugar production. On the basis of these findings Jost concluded that phospholipid is actively engaged, not only in the combustion of fatty acids, but also in the transformation of fatty acid into sugar. Attention may be called to the failure of other workers to observe either the increased sugar production by the phlorhizinized dog or the change in respiratory metabolism on injection of phospholipid intravenously (101, 226, 322).

Now, if phospholipid *is* continuously being synthesized by the liver and carried away by the blood stream to the other tissues, then these two organs should certainly show an active phospholipid turnover. It is significant therefore that Joannovics and Pick (125) had observed that in the liver of the dog the increase in the iodine number of the phospholipids caused by ingestion of cod liver oil had completely disappeared in 24 hours. A somewhat slower fall was found in the cat (260), the original level being reached only after two or three days. On the other hand, the fall in the iodine number of the phospholipids in the livers of rats was, like that of the muscle phospholipid, a very slow process (268), indicating again that the highly unsaturated acids within the phospholipids were being conserved. The same retention of fatty acids by the phospholipids is evident in the experiments of Artom (10, 11) who demonstrated for the first time that iodized fatty acids, whether injected intravenously or given by mouth, enter into the phospholipids of both the liver and the blood. Artom, himself, recognized that the amount of iodized fatty acids present did not diminish with time, but he regarded it of greater significance that ingested fat could be shown to have been transformed into phospholipid, presumably as a step toward its combustion.

We have then a definite lack of agreement between two lines of evidence bearing on phospholipid metabolism, the one from the study of changes in amount, the other from the study of changes in composition. When this lack of agreement has been satisfactorily explained away, a final answer can be given to the question as to whether or not the phos-

pholipids of liver and blood, in part at least, are intermediary products in the metabolism of fat.

## SUMMARY

Unlike the neutral fat of the body—which is clearly a fuel substance and therefore shows wide variations in amount—, the phospholipids are to be looked upon as an essential component of every tissue cell and, like the proteins, water and salts, are relatively constant in amount regardless of the nutritive state of the animal. Concerning their function, three possibilities must be borne in mind: 1, that they are intermediary products in fat metabolism, the replacement of one fatty acid of the triglyceride molecule by the phosphoric acid-base complex (as in the lecithins and cephalins), together with the desaturation of the fatty acids, being a means of rendering the fatty acids readily diffusible and combustible; 2, that they act as oxygen transport agents within the cells, alternately taking on and giving off oxygen at the unsaturated bonds of their fatty acids; 3, that they are concerned in some more strictly physicochemical sense in the structural make-up and activity of the cell.

Of these three theories, the first has enjoyed the greatest popularity and still is most widely accepted. In its favor may be cited the miscibility of the phospholipids in an aqueous medium, thus favoring their diffusion within the body; the high degree of unsaturation of the phospholipid fatty acids and therefore, according to the Leathes hypothesis, their readiness to undergo oxidation; the variations in the phospholipid content of the blood and to a less extent of the liver accompanying changes in the intensity of fat metabolism and transport; and the apparent entrance of unusual or entirely foreign ingested fatty acids into the phospholipid molecule.

On the other hand there are many well-established facts which are definitely not in line with the theory that the phospholipids are simply intermediary products in fat metabolism and which therefore form the basis for the two alternative theories of phospholipid function. The more outstanding of these facts are as follows: *a*. There is the very striking constancy in the amount of the phospholipid in the various organs—with the exception of the liver and especially the blood—in spite of sudden and extreme changes in the metabolism as a result of fat ingestion, hormone administration, intense muscular activity, and phospholipid injection. *b*. There is the fact that each organ of the body is characterized by a certain phospholipid content which has no apparent

relationship to the intensity of fat metabolism in that organ. Especially noteworthy is the higher phospholipid content of nerve tissue, especially the white matter; next in order come such organs as the liver and kidney; then come the skeletal muscles; and lastly comes the blood. *c.* There is the apparent antagonism between phospholipid and cholesterol, an antagonism which seems to be based on mutually opposite physicochemical properties. *d.* Then there is the peculiar relationship which has been shown to hold between the composition of the tissue phospholipids and the nature of the food fat. The high content of highly unsaturated, long-chain fatty acids within the tissue phospholipids; the readiness with which these long-chain fatty acids are taken up by the phospholipids when they are present in the diet; the tenacity with which these highly unsaturated acids are retained by the phospholipids when the supply has been cut off; the fixity in the ratio of saturated to unsaturated acids within the phospholipid molecule; all indicate that the high degree of unsaturation, so characteristic of the tissue phospholipids, is for some other purpose than to render them readily available for oxidative catabolism.

There are then many reasons for believing that the phospholipids of animal tissues fulfill some other function than that of intermediaries in the metabolism of fat. But whether that function is to transport oxygen by virtue of an easily reversible oxidation at the double bonds, or to contribute in some purely physical way towards cellular activity, must be left for future work to settle.

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# CAPILLARY PRESSURE AND CAPILLARY PERMEABILITY

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Until recently the capillaries were generally believed to be inert, thin-walled tubes serving to conduct blood through the tissues in whatever quantity the arterioles might supply. However, the monographs of Krogh (1922, 1929) and Lewis (1927) have summarized an impressive mass of evidence showing that the capillary vessels are independently contractile and capable of responding individually in a delicate manner to the circulatory needs of the immediately adjacent tissues. Even so, the great transporting medium, the blood stream, is still separated from almost all of the tissues it serves by a single layer of endothelial cells which form the walls of the capillary vessels. Obviously the interchange of substances between blood and the fluid bathing the tissues must depend fundamentally upon the properties of this membrane.

I. General nature of the capillary endothelium. The area of capillary wall available for fluid interchange is relatively enormous. Krogh (1922) estimates that if a man's muscles weigh 50 kgm. and his capillaries number 2,000 per square millimeter their total surface would be 6,300 sq. m. In the horse, dog and frog 1 cc. of blood is exposed in the capillary network to a filtering surface of 7,300, 5,600 and 2,700 sq. cm. respectively.

The capillary wall is also highly permeable since it permits fluid to pass much more easily than do other cellular membranes so far studied quantitatively. Strictly speaking, the permeability of a membrane must be defined in terms of units of volume or mass passing through unit area and thickness of membrane in unit time under the influence of unit hydrostatic or unit osmotic pressure. Using permeability so far as possible in this limited sense, the mesenteric capillary endothelium of the frog can be compared roughly with certain other cellular membranes. Lucké, Hartline and McCutcheon (1931) have shown that, under an osmotic pressure of one atmosphere, water passes through the membrane of the sea-urchin egg cell at the rate of 0.1 cubic micron per square micron of surface per minute. The membrane of the human erythrocyte is more

permeable (Jacobs, 1931); under an osmotic pressure of one atmosphere, water passes at the rate of 3.0 cubic micra (at 20°C.) per square micron of surface per minute. According to micro-injection experiments on single capillaries of the frog's mesentery (Landis, 1927b) a change in hydrostatic pressure of 5 cm. water modifies the filtration rate by 0.03 cubic micron per square micron of surface per second which corresponds to a filtration rate of 370 cubic micra per square micron of surface per minute under a pressure of one atmosphere. Thus fluid filters through the frog's capillary wall over 100 times more easily than through the membrane of the human erythrocyte and over 3000 times more easily than through the membrane of the sea-urchin egg cell. The importance of this high grade of permeability cannot be over-estimated in considering the physiology of the capillary network, one function of which is to distribute water and dissolved substances throughout the body efficiently and rapidly.

The significance of an extensive filtering area combined with great permeability is suggested by a calculation, which, on account of the assumption made, is admittedly more interesting than important. Assuming that the human capillary wall possesses a permeability to fluid similar to that of the frog's mesenteric capillaries, the total plasma volume of a man would be filtered through his calculated 6,300 sq. m. of capillary surface within 10 seconds at a capillary pressure of 10 mm. Hg if there were no force retaining fluid within the blood capillaries. Actually the vasomotor system normally prevents the entire peripheral vascular bed from opening simultaneously and, in addition, the colloidal constituents of blood plasma limit the loss of fluid from the blood stream. Only in widespread injury of the vascular endothelium would it be possible for fluid to leave the vascular system at such a rapid rate.

The movement of fluid between blood and tissue spaces varies in both rate and direction. Hydration, dehydration, change in posture and exercise affect the total volume and the water content of the blood but the variations are kept within safe physiological limits except under the most drastic conditions. Blood volume responds quickly to physiological needs and under pathological conditions shows great alterations, but the healthy organism strives to keep the general level constant (Erlanger, 1921).

Fluid absorbed from the gastro-intestinal tract dilutes the blood (Creveld and Feringa, 1921; Marx, 1925; Underhill and Sallick, 1925; Greene and Rowntree, 1927; Govaerts and Cambier, 1930; Bayliss and Fee, 1930; Rioch, 1930; Margaria, 1930; Farkas, 1932; Smirk, 1932;

Heller and Smirk, 1932a) during the period when the extra fluid is being transported throughout the body. Before diuresis begins some water passes into the tissues (Gömöri and Molnar, 1932; Heller and Smirk, 1932a) and, if excretion is delayed by administering pitressin (Heller and Smirk, 1932b), both hydremia and temporary storage in the tissues are accentuated. Limitation of water intake is accompanied by concentration of the blood (Underhill and Roth, 1922; Spencer, 1929; Kerpel-Fronius and Leövey, 1931) but the water content of the blood rapidly returns to normal when water is administered (Underhill and Kapsinow, 1922).

Muscular activity modifies the movement of fluid through the capillary wall; blood loses fluid rapidly during its passage through active muscle (Barcroft and Kato, 1915–16, and others) and lymph flows more rapidly from the region (White, Field and Drinker, 1933). During rest the loss of fluid from the blood ceases and lymph flow diminishes. Quiet standing diminishes blood volume (Thompson, Thompson and Dailey, 1927–28; Waterfield, 1931a; Youmans, Wells, Donley and Miller, 1933) and elevates the plasma protein concentration, while the lower extremities increase in volume (Waterfield, 1931b), owing to the extravascular accumulation of fluid which is filtered through the capillary wall. In the recumbent position this extravascular fluid is absorbed and blood volume returns to the earlier level. Under physiological conditions, therefore, fluid is moving at varying rates, at one moment inward, at another outward, through an extensive and highly permeable system of capillary walls. Nevertheless gross variations in blood volume and in the volume of tissue fluid are avoided.

To explain the equilibrium existing between blood and tissue fluid under these diverse conditions, the most attractive hypothesis is that advanced by Starling (1895–96). He pointed out that while the capillary endothelium is easily permeable to water and to solutions of most salts it is, in many tissues, relatively impermeable to the colloidal plasma proteins. The total osmotic pressure of the blood plasma amounts to approximately 7 atmospheres but is due almost entirely to substances which pass easily through the capillary wall. These crystalloids, being in a state of balance with the crystalloids in tissue fluid, cannot exert an osmotic pressure in the capillary and will not, under ordinary circumstances, influence the movement of fluid. The colloidal plasma proteins, on the other hand, are retained more or less completely by the endothelium and must therefore influence the movement of fluid through the capillary wall, though exerting a much smaller osmotic pressure,—found

by Starling to be about 30 mm. Hg. He suggested that although this colloid osmotic pressure is relatively insignificant, its order of magnitude is comparable to that of the probable capillary pressure. He postulated a balance between capillary pressure and the colloid osmotic pressure of the blood. In those areas where capillary pressure exceeds the colloid osmotic pressure of the blood, fluid will be filtered from the blood into the tissue spaces. In other areas where capillary pressure falls below the colloid osmotic pressure of the blood, fluid will be absorbed from the tissue spaces into the circulating blood.

This concept, in which physical forces become the prime agents activating the movement of fluid through the capillary wall, can be applied only if the endothelium of the capillary network acts like an inert membrane. From the morphological point of view it is not likely that a membrane having the structural simplicity of vascular endothelium could have a secreting function. The available evidence led Krogh (1929) to state, "When I review all the facts that have come to my notice I have no hesitation in saying that there is no trustworthy evidence of the capillaries having any power of hindering or favoring the passage by diffusion of all kinds of crystalloids through the endothelium." Recent chemical studies agree in demonstrating that at equilibrium electrolytes are distributed between blood serum and edema fluids in a manner which vigorously supports this view.

Mestrezat and Ledebt (1921) placed in the peritoneal cavities of animals collodion sacs filled with 0.5 per cent sodium chloride solution. After an interval of several days the fluid within the sacs resembled cerebrospinal fluid in freezing point, and in the content of chloride, sugar and protein, indicating that it is possible to obtain *in vivo* without "vital forces" an equilibrated dialysate which is superficially similar to at least one type of extravascular fluid.

Loeb, Atchley and Palmer (1922) compared ascitic fluid and blood, finding that the two fluids contained approximately equal amounts of $HCO_3$, Na, sugar, non-protein nitrogen and urea. The extravascular fluid differed from blood serum in its greater conductivity, its greater chloride and its lower potassium and protein contents. No new equilibrium was established when serum was dialyzed against the edema fluid through a simple collodion membrane. It was suggested that the relationship between serum and edema fluids results from a simple membrane equilibrium influenced in part by the amount of protein present.

Van Slyke, Wu and McLean (1923) recalculated the data of Loeb et al., expressing concentration in slightly different terms. The irregularity

of certain electrolyte ratios made it impossible to demonstrate quantitatively a Gibbs-Donnan equilibrium between blood serum and edema fluid. The agreement between the calculated and observed ratios for chloride, $HCO_3$ (arterial), and sodium seemed more than fortuitous and lent support to the concept that the capillary wall was a simple filtering membrane. This conclusion was verified by Hastings, Salvesen, Sendroy and Van Slyke (1925–27), who compared the electrolytes of edema fluid, ascitic fluid and blood serum. The $HCO_3$, Cl, Na, and H ratios approximated but still did not quite equal the ratios predicted on the basis of the Gibbs-Donnan equation. The K, Ca and Mg ratios departed widely from the predicted figure. Dialysis *in vitro* showed the same exceptions, however, and indicated that the discrepancies *in vivo* are to be attributed to physical factors inherent in the two solutions. Similar equilibria between plasma and edema fluid have been recorded by Muntwyler, Way and Pomerene (1931), Darrow, Hopper and Cary (1932b) and by Gilligan, Volk and Blumgart (1933) in various conditions. Schechter (1931) and Schechter et al. (1933) introduced into the peritoneal cavity solutions having an electrolyte composition more or less different from that of blood plasma and found that these extravascular fluids also attain eventually the pattern and distribution of electrolytes observed in spontaneously occurring edema fluids.

Greene and Power (1931) determined in dogs the distribution of electrolytes between arterial blood and an "*in vivo*" dialysate obtained through a collodion membrane. The distribution ratios thus obtained through a non-living membrane were then compared by Greene, Bollman, Keith and Wakefield (1931) with similar ratios between blood and extravascular fluids. The distribution of electrolytes in the system, blood plasma——capillary wall——extravascular fluid, is approximately the same as the distribution of these same ions in the system, blood plasma——collodion membrane——dialysate *in vivo*. The relative amount and the character of the protein present in the two solutions affects the distribution of the various ions between the serum and the transudate.

It cannot be said that the Gibbs-Donnan equilibrium holds accurately for any of these fluids but this is of no consequence in considering the properties of the capillary wall because those minor departures from theory observed in the case of edema fluids are also present in the ultrafiltrates or dialysates of plasma obtained *in vitro*. The essential point is that the electrolyte pattern observed when the capillary wall separates plasma and edema fluid is identical with the pattern observed when a

collodion membrane separates the same two fluids. It can be concluded safely, therefore, that the normal capillary wall does not act differently from a collodion membrane in this respect.

It is generally agreed that the slight differences between electrolyte concentration on the two sides of the capillary wall depend upon the difference between the protein contents of the two fluids. The equilibrium between the electrolytes of plasma and extravascular fluid is independent of the location of the edema fluid and also independent of the underlying pathological process, whether mechanical or inflammatory, which leads to the accumulation of tissue fluid (Loeb et al., 1922; Hastings et al., 1925). The distribution of electroytes is the same at the height, during increase, and during disappearance of edema (Muntwyler et al., 1931). There is no evidence that the capillary wall possesses a special permeability which leads to the accumulation of chloride in the extravascular fluid. Although the physico-chemical laws expressed in the Gibbs-Donnan equation are concerned in determining the composition of a transudate while it is in equilibrium with the blood stream, they will not explain either the development or the absorption of the transudate (Greene, Bollman et al., 1931; Muntwyler et al., 1931). The discrepancies *in vivo* from the Gibbs-Donnan equation can be explained for a complex system such as blood on the basis of physical factors (e.g., activity coefficients of the ions) inherent in the two solutions, particularly since similar discrepancies are observed *in vitro* (Ingraham, Lombard and Visscher, 1933). Moreover, it has been pointed out (Greene, Bollman et al., 1931; Darrow, et al., 1932b) that it is difficult to obtain a sample of blood truly representative of that with which the particular transudate is in equilibrium. Greene, Bollman et al., (1931) mention that, in patients who had been taking ammonium nitrate, the nitrate content of the ascitic fluid might presumably lag behind that of serum, both when the amount in serum is increasing during administration and when the amount in serum is decreasing after administration has been stopped. With circulatory and chemical changes constantly taking place, large volumes of transudate probably cannot, except by chance, be in absolute equilibrium with any one blood sample.

From the physico-chemical standpoint the capillary wall can be regarded as an ultrafilter comparable to an artificial membrane impermeable to protein. It follows therefore that the osmotic pressure of the proteins must tend to absorb water from the tissue spaces, and that this absorbing force cannot be balanced by any other force than capillary blood pressure. Peters (1933) holds that the distribution of electrolytes

between blood and extravascular fluid supports Starling's theory just as adequately as the available physiological evidence.

*Summary.* The general properties of the capillary wall include 1, an enormous total area for interchange between the blood and tissue spaces; 2, permeability to fluid which is many times greater than that of certain cell membranes so far studied quantitatively, and 3, the physical characteristics of an inert (in the sense of non-secreting) membrane permeable to water and crystalloids but relatively impermeable to the plasma proteins. With this background it is justifiable,—or even obligatory,—to regard physical forces as important factors in the movement of fluid through the capillary wall. It is also necessary to examine critically so-called "changes in capillary permeability" in which these physical forces have not been carefully controlled.

II. PHYSICAL FACTORS AFFECTING THE MOVEMENT OF FLUID THROUGH THE CAPILLARY WALL. *1. Capillary blood pressure.* Blood flows past the highly permeable capillary wall under a hydrostatic pressure. Theoretically this capillary blood pressure would be expected to filter fluid from the blood through the capillary wall into the extravascular spaces. Capillary pressure interested early investigators of hemodynamics primarily because of its relation to the location of the peripheral resistance to blood flow. Fick (1888) placed practically the whole of the peripheral fall in blood pressure on the venous side of the capillary network. Since capillary pressure, according to some determinations, was extremely low, Krogh (1922) held that the peripheral fall in blood pressure must be situated primarily on the arterial side of the capillary network, a drop of a few millimeters of water pressure being sufficient to overcome the frictional losses of energy required to produce capillary blood flow. An intermediate view was expressed by Levy (1897) and by Dale and Richards (1919) who believed that the peripheral resistance was situated partly in the arterioles, and partly in the capillaries. This latter view postulates a gradient of pressure in the capillaries themselves. The validity of Starling's hypothesis depends, among other things, on the presence of such a gradient and on the demonstration that an excess of capillary pressure over the colloid osmotic pressure of the blood actually filters fluid from the capillary blood. The measurement of capillary blood pressure is of theoretical importance also in determining whether this force and the colloid osmotic pressure of the blood are in approximate balance.

a. *Methods of measuring capillary blood pressure.* Capillary blood pressure has been measured directly (Landis, 1926, 1930b) and, far more

frequently, indirectly. The many indirect methods of measuring capillary pressure are fundamentally similar; they consist in applying graded pressure to a tissue either by means of a transparent rigid plate, or by means of a capsule covered by a distensible, transparent membrane. They differ considerably in the arbitrary criterion taken to indicate a state of balance between intravascular pressure and external pressure. The criteria which have been used are extremely numerous, including, to mention only a partial list: 1, first perceptible blanching of the skin; 2, definite or even complete blanching of the skin; 3, appearance of capillary pulsation in a histamin flare; 4, blanching with only brief pressure by a jet of water; 5, return of skin color in a histamin flare to normal depth of color; 6, complete stoppage of flow in capillary vessels viewed under the microscope; 7, first perceptible modification of flow in single capillaries, and 8, the disappearance and reappearance of the spectroscopic bands characteristic of hemoglobin.

It is not surprising that little agreement exists in the results obtained in accordance with these various criteria. Table 1 collected by Strax and De Graff (1931) summarizes the capillary blood pressure measurements in man. The average normal values reported for a single tissue, skin, vary over a range so wide (1.5 to 71.0 mm. Hg) that it is impossible to draw any definite conclusion concerning the relation between capillary pressure and the colloid osmotic pressure of the blood. The same method, used by several investigators, yields values differing by several hundred per cent. This is to be expected from the subjective nature of the criteria adopted, particularly where fine gradations of skin color are concerned.

Danzer and Hooker (1920) and Liebesny (1923) found that external pressure blanches the skin by emptying the subpapillary venous plexus and not the capillaries. According to White (1924b) the pressure required to produce blanching has no relation either to capillary or to venous pressure, being occasionally even lower than venous pressure. Moreover variations from subject to subject are due largely to differences in normal skin resistance, a factor involved in all indirect methods. Blockage of circulation by long-continued external pressure has been criticized by Hill and McQueen (1928) and is undoubtedly responsible for some of the very high figures reported. On the other hand, mere modification of blood flow in a few capillaries, the criterion advocated by Hill (1920), can be produced by pressures below venous pressure (Landis, 1926).

The wide range of the indirectly determined estimates of capillary

blood pressure is doubtless due to errors which arise from the arbitrary adoption of different methods and criteria by almost every investigator.

TABLE 1

*Capillary pressure determinations in man (modified from Strax and De Graff, 1931)*

| | | mm. Hg |
|---|---|---|
| I. Indirect methods: | | |
| (a) With skin blanching: | | |
| 1. von Kries | 1875 | 24–38 |
| Natanson (v. Kries method) | 1886 | 71 |
| 2. v. Basch | 1900 | 25–30 |
| 3. von Recklinghausen | 1906 | 55 |
| Krogh and Rehberg (von Recklinghausen method) | 1927 | 5–8 |
| 4. Basler | 1912 | 7–9 |
| Landerer (Basler method) | 1913 | 17–25 |
| Goldmann (Basler method) | 1914 | 5–7 |
| 5. Marks | 1920 | 6–20 |
| 6. Hill and McQueen | 1921 | 10 |
| 7. Ariola | 1925 | 10–14 |
| 8. Ellis and Weiss | 1929 | 6–12 |
| (b) With direct visualization of capillaries: | | |
| i. Pressure applied by rigid surface: | | |
| 1. Lombard | 1911–12 | 30–45 |
| 2. Guillaume | 1924 | 18–28 |
| 3. Rajka | 1926 | 1–2 |
| 4. Strax and De Graff | 1931 | 18–29 |
| ii. Pressure applied by elastic capsule: | | |
| 1. Krauss | 1914 | 10 |
| 2. Danzer and Hooker | 1920 | 20–24 |
| Boas (Danzer and Hooker method) | 1922a | 20–30 |
| 3. Kylin | 1921 | 13.6 |
| Rominger (Kylin method) | 1923 | 8 |
| Liebesny (Kylin method) | 1923 | 32.5 |
| Nevermann (Kylin method) | 1924 | 6–11 |
| Grzechowiak (Kylin method) | 1924 | 6–14 |
| (c) Bleeding methods: | | |
| 1. Basler | 1914 | 7.7–13.0 |
| 2. Weiss | 1914 | 10 |
| II. Direct methods: | | |
| (a) Intubation, brief periods, Carrier and Rehberg | 1923 | 3.3–5.5 |
| (b) Intubation, long periods, Landis | 1930b | 12–32 |

Depending on selection of figures in table 1 capillary pressure in man seems to be far below or far above the colloid osmotic pressure of the plasma proteins and no conclusions can be drawn concerning the balance

between these two forces. Moreover, no indirect method can yield exact information concerning the existence or non-existence of a gradient of pressure in the capillary network itself. These difficulties led to direct determinations through minute cannulae inserted into single capillaries (Carrier and Rehberg, 1923; Landis, 1926).

A "direct" measurement of arterial or venous blood pressure requires that a cannula be inserted into the vessel and retained there long enough to secure complete balance between the blood in the vessel and the fluid in the manometer. A "direct" measurement of capillary pressure should have the same qualifications. Thus determining the pressure required to prevent bleeding after an incision is made in the skin is not strictly speaking a direct method, though often so termed. Basler (1914) and Weiss (1914) found that relatively low pressure sufficed to stop bleeding produced in this manner. There is no assurance that only capillaries have been incised, nor is it certain that applying an anticoagulant to the surface of the skin prevents clotting in the deeper parts of the puncture. Blood will finally cease flowing spontaneously without the application of any external pressure.

The first measurements of human capillary pressure by an intubation method were those of Carrier and Rehberg (1923) who observed the pressure at which blood would, or would not, flow into the tip of a pipette held in the hand and introduced into a capillary loop. The size of their pipettes restricted measurements to venous loops and the lack of rigid support for the pipette limited the period of observation to a few seconds. Kylin (1926) offered the objection that introducing a glass tube into the venous limb obstructs inflow from the arterial side. Under these circumstances, according to Krogh (1929), the venous limb and the minute cannula form a side tube to the nearest venule and the pressure measured is then that in the first net of venules instead of that at the top of the capillary loop. Of equal importance is the repeated observation (Landis, 1930b) that to draw blood rapidly into a fine micropipette requires a pressure approximately 5 to 10 mm. Hg lower than true equilibrium pressure. Pressure measurements should be made (Landis, 1926) only at true equilibrium when no fluid is moving through the tip of the pipette, since an orifice of 8 to 15 micra interposes considerable resistance to flow. Even pulsatile movement of the blood in the pipette is not evidence of equilibrium unless the corpuscles oscillate about the same point for 5 to 10 seconds without continuous movement either into, or out of, the pipette. Hence, it would seem that a true "direct" measurement of capillary pressure, like the "direct" measurement of arterial pressure,

should continue for a period which is long enough to ensure a complete balance of pressure between capillary blood and the fluid communicating with the manometer.

The direct micro-cannulation method used by Landis (1926, 1930a, 1930b) has the advantage of permitting *a*, repeated measurements over long periods of time, and *b*, pressure determinations in various parts of the capillary network and larger vessels. It is thus possible to follow variations in capillary blood pressure from moment to moment and also to determine the gradient of blood pressure in the capillary network itself. Micropipettes, their movement governed by a Chambers micromanipulator, are inserted into individual capillaries. The other end of the micropipette is connected with a water or mercury manometer and with a device for changing pressure within these pipettes and the manometer system. When equilibrium is established corpuscles oscillate freely with the pulse but move neither toward nor away from the tip of the pipette. Blockage of flow is to be carefully avoided to prevent the recording of erroneously high values. This procedure with due precaution fulfills the requirements outlined above for a true "direct" measurement and provides results which are most nearly free from criticism (Krogh, 1929; Strax and De Graff, 1931; Drinker and Field, 1933). Skin resistance and the danger of obstruction to flow are eliminated. When measurements are first made a transitory rise of pressure owing to stimulation of the axone reflex sometimes appears, but this lasts only a few minutes after which a lower, and presumably normal, capillary pressure can be measured repeatedly over extended periods. Ellis and Weiss (1929) have criticized the use of nail bed capillaries,—the only easily accessible ones in man,—on the basis of possible abnormalities because of exposure to light and variations in temperature. The direct method is not suited to clinical measurements since only a few capillaries can be pierced in the course of an hour and averages are unjustified unless large numbers of determinations have been made.

Fewer indirect determinations of capillary blood pressure have been made in lower animals and the results are discordant. Roy and Brown (1875) used a capsule with a distensible membrane and obstructed flow completely. They recorded relatively high pressures in the capillaries of the frog's web, tongue and mesentery. Hill (1921a, 1921b) used the same apparatus but regarded the true capillary pressure to be that pressure which, when suddenly applied, momentarily modified, but did not stop, capillary flow. He recorded pressures in the web, mesentery and tongue of the frog which were only one-third to one-sixth as great as

those recorded by Roy and Brown,—lower in fact than the venous pressures observed by direct methods (Landis, 1926). The wide discrepancy in the results obtained with the same apparatus, and in the same animal, emphasizes again the purely arbitrary nature of the criterion of balance between extravascular and intravascular pressures when measured indirectly. Micro-cannulation (Landis, 1926, 1931) indicated that average capillary pressure in the frog lies between these two extremes. This direct method, though carried out without obstruction to flow, has been criticized on the ground that the tissues investigated are exposed and abnormal. However, injury of the skin (Landis, 1930b) and tetanus of muscle elevates capillary pressure well above the normal level (Landis, 1931) so that the permanent effects of exposure cannot be very great.

*Summary.* The indirect methods of measuring capillary blood pressure have yielded figures which vary so widely that it is impossible to draw conclusions concerning either the absolute level or the gradient of capillary blood pressure. The direct micro-injection method, though technically difficult, yields more exact information because there is less doubt concerning the pressure required accurately to balance the blood pressure in single capillaries. The direct method also permits determining the gradient of blood pressure in the peripheral vessels.

b. *The average gradient of capillary blood pressure.* In agreement with Levy (1897) and Dale and Richards (1919) it was found in the mesentery (Landis, 1926), web and muscle (Landis, 1931) of frogs, in the mesentery of the rat and guinea pig (Landis, 1930a) and in the skin of man (Landis, 1930b) that the fall in blood pressure does not cease at the junction of the arterioles and capillaries but continues through the capillary network. In exposed tissues 20 to 30 per cent of the peripheral resistance to blood flow is situated in the capillaries. As shown in figure 1 the blood pressure decreases but slightly in the larger arteries; the gradient is steepest in the arterioles, but continues through the capillaries. The four curves are similar in form, differing, however, in the levels at which blood pressure is maintained.

A fall of pressure amounting to only a few millimeters of water is, in fact, not enough to produce the average rates of flow observed in the frog's mesentery. Cinematographic recording of the flow of an opaque substance through the frog's mesenteric capillaries indicated that the drop in capillary pressure cannot be less than 1.0 to 2.0 cm. of water and is considerably higher when allowance is made for theoretical difficulties encountered in applying Poiseuille's equation to the capillary circulation (Landis, 1933). The results of calculation and direct observation agree

in indicating that arteriolar capillary pressure is higher than venous capillary pressure by a significant amount.

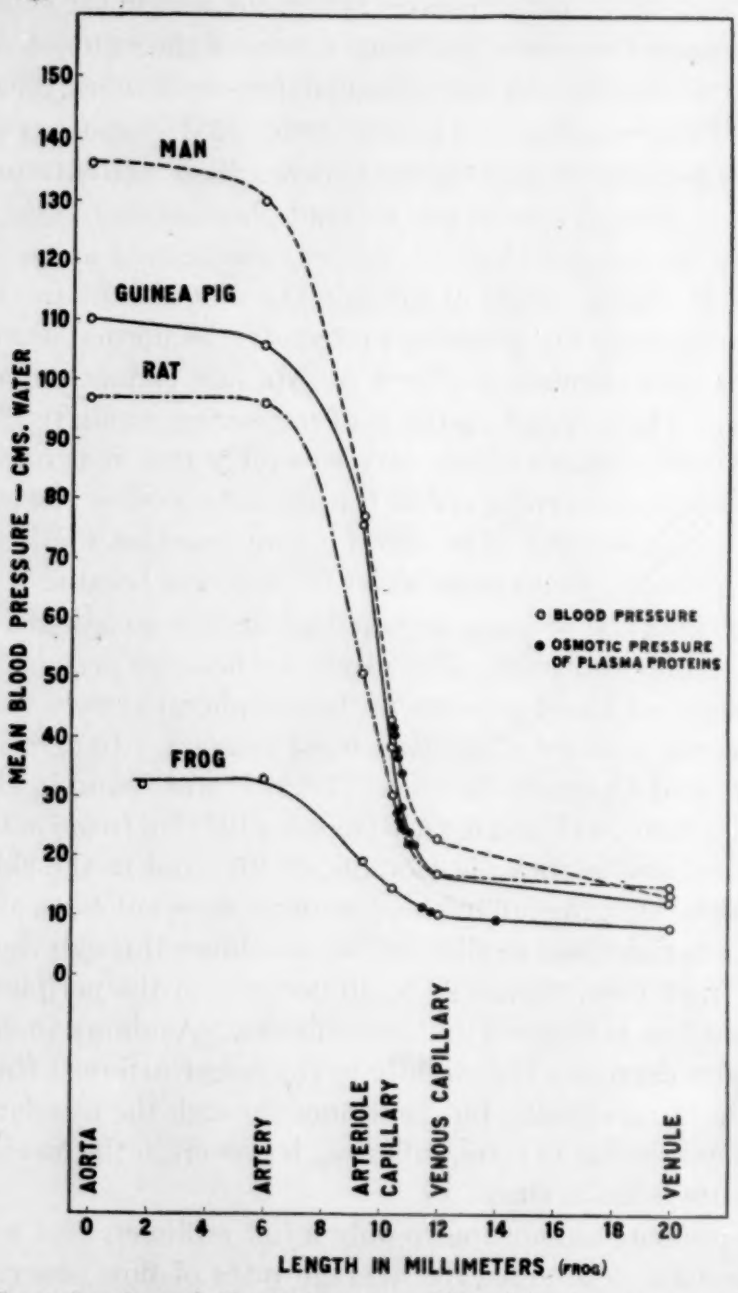


Fig. 1. Chart showing relation between capillary blood pressure and the osmotic pressure of the plasma proteins in four species.

In the frog, for instance, average arteriolar capillary pressure is 14 cm. of water while venous capillary pressure is 10 cm. of water. In man the arterial capillary pressure amounts, on the average, to 45 cm. of

water, venous capillary pressure to about 22 cm. of water. The blood colloid osmotic pressures of these two species show corresponding differences (table 2). The normal colloid osmotic pressure of frog plasma has been variously reported to range from 5.5 to 13.4 cm. of water while the colloid osmotic pressure of human blood amounts to about 36 cm. of water. The rat and guinea pig occupy an intermediate position with regard to both capillary pressure and the colloid osmotic pressures of

TABLE 2

*Blood colloid osmotic pressure and capillary pressure (micro-injection determinations)*

| ANIMAL | COLLOID OSMOTIC PRESSURE OF BLOOD | AVERAGE BLOOD PRESSURE | | TISSUE |
|---|---|---|---|---|
| | | Arteriolar capillary | Venous capillary | |
| | *cm. water* | *cm. water* | *cm. water* | |
| Frog | 5.5– 6.0 (Krogh, 1922) | 14.4 | 10.1 | Mesentery (Landis, 1926) |
| | *6.8–13.4 (Churchill, Nakazawa and Drinker, 1927) | 14.9 | 9.5 | Muscle (Landis, 1931) |
| | †9.6–11.5 (White, 1924a) | 14.5 | 10.0 | Skin (Landis, 1931) |
| | 11.7 (Landis, 1927b) | | | |
| Rat | 22.0–26.5 (Landis, 1930a) | 30.0 | 17.0 | Mesentery (Landis, 1930a) |
| | 24.0–29.0 (Meyer, 1932) | | | |
| Guinea pig | 22.5–27.5 (Landis, 1930a) | 38.5 | 17.0 | Mesentery (Landis, 1930a) |
| | 24.0–25.0 (Kylin and v. Pein, 1931) | | | |
| | 25.0–28.0 (Meyer, 1932) | | | |
| Man | 33.0–40.0 (Starling, 1895–6) | 43.5 | 16.5 | Skin, base of nail (Landis, 1930b) |
| | 35.0–40.0 (Govaerts, 1924) | | | |
| | 28.5–36.5 (Schade and Claussen, 1924) | | | |
| | 33.0–40.0 (Verney, 1926) | | | |
| | 29.5–39.0 (Krogh and Nakazawa, 1927) | | | |
| | For others see Meyer (1932) | | | |

* Normal frogs only.

† Cm. plasma.

their bloods. The gradient of capillary pressure in at least four species is such that on the average arteriolar capillary pressure is above the osmotic pressure of the plasma colloids while average venous capillary pressure is below the osmotic pressure of the plasma colloids (fig. 1; table 2). It is physically possible, therefore, that on the average, fluid is filtered from blood during its passage through the arteriolar portion of the capillary network while absorption takes place in the venous portion of the capillary network. Though this represents the average condition,

fluid movement in individual capillaries will diverge from the average in accordance with variations in capillary pressure, diameter and flow.

*Summary.* The peripheral fall in blood pressure does not cease at the junction of the artericles and capillaries, but continues through the capillary network. In four species studied by the micro-cannulation method, average capillary pressure is approximately equivalent to the colloid osmotic pressure of the plasma proteins. Owing to the gradient of capillary pressure, under average conditions filtration is favored in

TABLE 3

*Variations in capillary pressure*

| | CAPILLARY PRESSURE | | | | | |
|---|---|---|---|---|---|---|
| | Arteriolar capillary | | | Venous capillary | | |
| | Highest | Lowest | Average | Highest | Lowest | Average |
| | *cm. water* | *cm. water* | *cm. water* | *cm. water* | *cm. water* | *cm. water* |
| Frog, mesentery | 22.0 | 5.0 | 14.4 | 18.0 | 6.7 | 10.1 |
| web, normal. | | | | | | |
| urethane | 19.0 | 10.0 | 13.9 | 13.0 | 8.5 | 9.6 |
| curare | 20.5 | 10.0 | 14.5 | 15.5 | 8.5 | 10.0 |
| web, hyperemia | 26.5 | 14.0 | 19.5 | 17.5 | 15.0 | 16.5 |
| muscle, normal | 18.0 | 11.0 | 14.9 | 12.7 | 7.0 | 9.5 |
| hyperemia | 26.0 | 17.0 | 20.1 | 17.5 | 12.0 | 16.0 |
| Rat, mesentery | 34.0 | 22.0 | 30.0 | 20.0 | 15.0 | 17.0 |
| Guinea pig, mesentery | 49.0 | 31.0 | 38.5 | 19.5 | 13.0 | 17.0 |
| Man, skin, normal | 65.0 | 28.6 | 43.5 | 24.5 | 8.0 | 16.5 |
| hyperemia | 93.0 | 71.0 | | 66.5 | 54.5 | |

the arteriolar portion of the capillary network, while absorption is favored in the venous portion of the capillary network.

c. *The variability of capillary blood pressure.* The capillary pressures recorded in table 2 and in figure 1 represent averages of numerous single determinations. Capillary pressure is, however, an extremely variable quantity, changing in the same capillary from moment to moment and differing widely in adjacent capillaries connected to the same arteriole (Roy and Brown, 1875; Landis, 1926). Table 3 shows that these variations, spontaneous or induced, are so large that blood pressure in an

entire capillary, or even in a whole network, may be at one moment above, at another below, the colloid osmotic pressure of the blood.

Arteriolar constriction increases the peripheral resistance and then capillary pressure becomes lower throughout the affected area, irrespective of capillary diameter. Pulse pressure in the capillary network decreases while the gradient of blood pressure becomes steeper in the arterioles and flatter in the capillaries. These changes were observed by Landis (1926) in the frog's mesentery after hemorrhage and during constriction produced by adrenalin. Raynaud's disease in man is a condition characterized by arterial spasm followed by arterial relaxation and reactive hyperemia. During spasm capillary pressure is low,—7 to 8 mm. Hg—and pulse pressure is small, while during arterial relaxation capillary pressure may reach 40 mm. Hg with a large pulse pressure (Landis, 1930c).

Dilatation of the arterioles shifts the peripheral fall of blood pressure toward the capillary network, elevates capillary pressure, and increases the pulse pressure in the capillary vessels. These effects were observed by direct measurement during hyperemia of the frog's mesentery (Landis, 1926), muscle and skin (Landis, 1931). In man cutaneous capillary pressure rises during hyperemia produced by heat (Goldmann, 1914; Danzer and Hooker, 1920; Lewis and Haynal, 1928; Landis, 1930b), histamin (Lewis and Haynal, 1928; Landis, 1930b) and acute inflammation (Landis, 1930b). In every instance blood flow becomes more rapid and pulse pressure increases. The appearance of capillary pulsation in normal individuals after heating the skin (Lewis, 1924) is in accord with these findings, the pulse penetrating even to the subpapillary venous plexus (Boas, 1922b). The changes in capillary pressure accompanying arteriolar constriction and dilatation verify the conclusions of Bayliss and Starling (1894) who pointed out the fallacy of arguing that capillary pressure must necessarily follow arterial pressure. Adrenalin elevates arterial pressure but lowers capillary pressure. Vasodilatation, if widespread, lowers arterial and capillary pressures but may elevate capillary pressure locally if systemic blood pressure is not reduced too greatly.

When capillary pressure is elevated by arteriolar dilatation, whether spontaneous or induced, the rate of capillary blood flow is increased, the relation between flow and pressure being often extremely close (Landis, 1926). Capillary blood flow, while usually constant, often shows rhythmic increase in velocity during cardiac systole (Hürthle, 1923). This pulse, like pulse pressure measured directly (Landis, 1930b), penetrates at times to the venous capillaries.

A rise of venous pressure elevates capillary pressure (Danzer and Hooker, 1920; Liebesny, 1923; Carrier and Rehberg, 1923; Lewis and Haynal, 1928; Landis, 1930b) even when the obstruction involves only a few veins or venules (Landis, 1926). If the veins of the upper arm are compressed by a pneumatic cuff of sufficient width capillary pressure equals or slightly exceeds the pressure in the cuff within 15 to 45 seconds. It is impossible for capillary pressure to be below venous pressure except for very brief periods. The effects of the Valsalva experiment on capillary pressure (Danzer and Hooker, 1920; Landis, 1930b) can be ascribed to the rise of venous pressure.

Likewise capillary pressure is high in dependent parts of the body owing primarily to the hydrostatic pressure of the blood in the vertically placed veins. Carrier and Rehberg (1923) and Landis (1930b) found that when the hand is above the base of the heart capillary pressure remains at a low but constant level in all positions. The discrepancy in the absolute pressures observed is to be ascribed to the brief periods of cannulation used by Carrier and Rehberg. Both sets of figures agree in showing that when the hand is dropped below the heart the increase of capillary pressure is almost identical with the theoretical increment due to the hydrostatic pressure of the column of venous blood. Thus according to Landis (1930b) if the hand is held quietly 30 cm. above the base of the heart capillary pressure in the arteriolar and venous capillary limb falls below the colloid osmotic pressure of the blood. If the hand is 40 cm. below the heart, pressure, even in the venous capillary, is above the colloid osmotic pressure of the blood.

Local heating produces hyperemia and elevates capillary pressure (Danzer and Hooker, 1920; Lewis and Haynal, 1928; Landis, 1930b), while cold diminishes capillary pressure during the first few minutes (Danzer and Hooker, 1920; Landis, 1930b), though when reactive hyperemia sets in capillary pressure may rise (Landis, 1930b). Diurnal variations in capillary pressure may or may not exist (Danzer and Hooker, 1920; Kylin, 1926). Innervation affects capillary pressure which rises when the axone reflex is stimulated (Landis, 1930b, 1931). In hemiplegia blood flow is rapid in the affected extremity and capillary pressure high (Weiss and Ellis, 1931).

*Summary.* Capillary pressure is extremely variable; its changes cannot always be predicted by measuring arterial pressure. The height of capillary pressure depends upon arterial tone, freedom of venous outflow, posture and temperature,—to mention only those factors which have been studied in detail. Blood pressure in an entire capillary, or

even in a whole network, may be at one moment far above, at another far below, the colloid osmotic pressure of the blood, favoring massive filtration or massive reabsorption, respectively, over large areas of endothelium.

d. *Capillary blood pressure and fluid movement.* The indecisive results of older experiments on the qualitative changes in fluid movement produced by changes in arterial pressure have been reviewed by Oehme (1928). Elevating arterial pressure for very brief periods of time by compressing the aorta is not accompanied by loss of fluid from the blood, according to Tani (1924), but Adolph and Lepore (1931) were able to detect an increase in the water content of the tissues. Lymph flow is not increased by elevating arterial pressure until very high levels are reached, when arteriolar resistance appears to break down (Haynes, 1932a). Scott, Rabinowitz and Rupp (1923) state, on the other hand, that elevating systemic blood pressure by injecting adrenalin increases the concentration of Congo red in the plasma, and conclude that fluid leaves the blood stream, the colloidal dye leaving more slowly than fluid. The frequent independence of arterial and capillary pressures probably explains the varying results.

Active hyperemia elevates capillary pressure and would therefore be expected to facilitate filtration. Stimulating the lingual nerve produces active hyperemia of the tongue, but edema appears regularly only in the presence of hydremic plethora (Starling, 1894). It must be remembered, however, that tissue volume must be increased by 10 per cent before edema is grossly detectable (Drury and Jones, 1927). The active hyperemia accompanying heating of the skin favors the passage of dyes from the blood stream (Okuneff, 1924; Anitschkov, 1924; Hudack and McMaster, 1932) but damage to the capillary wall is also a possible factor. Nerve section produces more general staining by intravenously administered dye (McMaster, Hudack and Rous, 1932) but does not affect lymph production in the dog's leg (Haynes, 1932a). Vasodilator drugs increase the conjunctival edema produced by mustard oil only when they also raise capillary pressure; the vasodilator drugs lose their effect on fluid movement when systemic blood pressure falls (Hirschfelder, 1924). The active hyperemia which accompanies functional activity is complicated by metabolic factors and will be considered in a later section.

Arteriolar constriction produced by adrenalin or by cocaine diminishes the conjunctival edema produced by mustard oil (Hirschfelder, 1924). The loss of fluid in paraphenylenediamine edema is diminished by a drop

in systemic blood pressure or by the administration of adrenalin (Tainter and Hanzlik, 1924). The injection of pituitrin, which increases vascular tone in mammals, diminishes lymph flow and delays the exit of salt solution from the blood stream according to Bayley et al. (1925). Hemorrhage which reduces capillary blood pressure (Landis, 1926) withdraws fluid from the tissue spaces of the dog at the rate of 0.25 cc. per kilo of body weight per minute corresponding to a (calculated) fall in capillary pressure of 28 cm. water (Adolph, Gerbasi and Lepore, 1933). The effects of arterial tone on fluid movement are therefore not absolutely uniform, but in general vasodilatation appears to favor filtration while vasoconstriction appears to hinder filtration.

The qualitative effects of elevating venous pressure, and therefore capillary pressure, are more uniform. Lymph flow from the thoracic duct increases when abdominal venous pressure is raised (Starling, 1894). Similarly subcutaneous lymph flows more rapidly during venous congestion (White, Field and Drinker, 1933) and when the limb is dependent. Venous congestion elevates the protein and hemoglobin concentration of the blood (Yamaguchi, 1927a, b; Plass and Rourke, 1927) roughly in proportion to the venous pressure (Landis, Jonas, Angevine and Erb, 1932). Venous congestion, according to McMaster, Hudack, and Rous (1932) increases also the amount of dye passing into the tissue spaces after intravenous injection. Postural increase of venous pressure concentrates the plasma proteins generally (Thompson, Thompson and Dailey, 1927–28; Waterfield, 1931) and particularly locally in the vessels of dependent tissues (Youmans et al., 1933). The volume of the leg increases owing to the accumulation of fluids in the tissue spaces (Waterfield, 1931b). Partial obstruction of the vena cava leads to ascites even in the presence of normal lymphatic drainage (Bolton, 1924). Perfusing the dog's leg with defibrinated blood produces edema if venous pressure is above 10 mm. Hg; at venous pressures below this no true edema develops though a small amount of fluid leaves the blood (Bornstein and Budelman, 1930). Loss of fluid from blood by filtration is detected consistently therefore when capillary pressure is elevated by raising venous pressure. These observations show, however, only a qualitative relationship between hydrostatic pressure and filtration. Other things remaining constant it would be expected, in the presence of an inert capillary wall, that filtration must be directly proportional to the hydrostatic pressure exerted upon the capillary blood.

Micro-injection experiments on single capillaries in the frog's mesentery (Landis, 1927b) indicate that in this animal and tissue, when the hydro-

static pressure falls below 9 cm. of water, fluid moves from the extravascular spaces into the blood. When capillary pressure is above 12.5 cm. of water fluid moves from the blood to the tissue spaces. The rate of absorption and the rate of filtration are roughly proportional to the difference between capillary pressure and 11.5 cm. of water. When the rate of filtration or absorption is charted against capillary pressure a straight line can be drawn through the points, indicating that the rate of fluid movement, when studied in single capillaries, is directly proportional to hydrostatic pressure.

In normal human subjects Mende (1919) observed the effect on limb girth of raising venous pressure by means of a narrow rubber cuff inflated to various pressures. He concluded that a venous pressure of 50 cm. was required to produce the first measurable filtration of fluid. This method of identifying increase in limb volume is so rough that small accumulations of fluid might easily have been overlooked. Drury and Jones (1927) measured the rate of filtration in the lower extremities by means of a water plethysmograph during venous congestion. They found that the rate of filtration was roughly proportional to the increase in venous pressure. They were not able, however, to measure filtration during the first ten minutes of venous congestion on account of the time required for the congested veins to fill completely with blood. Krogh, Landis and Turner (1932) used a "pressure plethysmograph" by means of which volume changes due to vascular tone were eliminated during the time that limb volume was being measured. In this way small accumulations of fluid were estimated and filtration rates were measured even during the first minutes of venous congestion. No filtration of fluid could be detected by this method until venous pressure was above 15 cm. of water. Later observations by Landis and Gibbon (1933) with still another type of "pressure plethysmograph" yielded essentially the same result. When venous pressure in the forearm was elevated to various levels above this so-called "margin of safety" the rate of filtration was directly proportional to the increase in venous pressure, at least up to the highest pressure tested, 80 cm. of water. The change in the rate of filtration accompanying unit rise (1 cm. of water) in venous pressure amounted to between 0.0023 and 0.0028 cc. per minute per 100 cc. of forearm when the congestion periods were thirty minutes long (Krogh et al., 1932; Landis and Gibbon, 1933) and to 0.0033 cc. per minute per 100 cc. of forearm when the congestion periods were ten minutes long (Landis and Gibbon, 1933).

*Summary.* The movement of fluid from blood to tissue spaces varies

considerably with circulatory adjustments and particularly with capillary pressure. High arterial pressure and active hyperemia usually, but not always, increase the filtration of fluid. Elevating venous pressure is always associated with increased filtration, the rate of filtration being proportional, within certain limits, to the venous pressure.

2. *The colloid osmotic pressure of the blood.* Though Graham (1861) mentioned that the plasma colloids might exert an osmotic pressure in the vascular system, it remained for Starling (1896) to measure the magnitude of this force in the blood plasma. This subject, reviewed in detail by Meyer (1932), will be considered here only in its relation to the movement of fluid and dissolved substances through the walls of the systemic capillaries.

The balance between colloid osmotic pressure and capillary blood pressure (Landis, 1926, 1930a, c) offers presumptive evidence that the plasma proteins aid in controlling fluid movement. The association of a low colloid osmotic pressure of the blood with certain forms of edema occurring as a result of disease in man (Krogh, 1922; Govaerts, 1924; Schade and Claussen, 1924; and others) also suggests, but does not prove conclusively, that movement of fluid through the capillary wall is related to the colloid osmotic pressure of the blood. In dogs Leiter (1928, 1931) showed that chronic hypoproteinemia produced by plasmapheresis leads to the accumulation of extravascular fluid with outspoken edema when the plasma protein falls to 3 per cent or less. The relation between hypoproteinemia and edema was verified in plasmapheresis by Shelburne and Egloff (1931), Lepore (1931, 1932), Darrow, Hopper and Cary (1932a, b), Weech, Snelling and Goettsch (1933), and in experimental malnutrition by Kohman (1920), Frisch, Mendel and Peters (1929), Shelburne and Egloff (1931), and Weech, Snelling and Goettsch (1933). Even in the presence of a low plasma protein, however, edema cannot develop without an adequate supply of salt and water (Leiter, 1928; Fahr, Kerkhof and Giere, 1931; Weech, Snelling and Goettsch, 1933). Clinical experience in the treatment of edema has shown empirically that a physical "tendency toward edema" can be neutralized by restricting the intake of salt and water or by increasing the excretion of water and salt by means of diuretics. Plasmapheresis also increases lymph flow considerably (Field and Drinker, 1931a) and a low plasma protein favors the movement of dye into the tissue spaces (Smith and Dick, 1932).

Drinker (1927) observed that the addition of relatively large quantities of horse serum to the perfusing fluid prevents subcutaneous edema from

appearing in the perfused leg of the frog. He concluded that the force which retains fluid within the blood vessels is associated with the protein, but found no evidence that the favorable effect of serum is due to the colloid osmotic pressure of the perfusing fluid. The large amount of protein required in Drinker's experiments may, however, be related to the greater permeability of the capillary walls of the frog, particularly after perfusion (Conklin, 1930b) of the skin vessels. Leakage of protein would diminish the effective colloid osmotic pressure to a point considerably below the colloid osmotic pressure measured *in vitro*. Under these conditions higher concentrations of protein would be required to prevent edema. Kitmura (1927) states that the effect of colloids upon the accumulation of edema fluid in the perfused frog's leg depends upon whether the colloid is, or is not, retained in the blood vessels.

Abnormal filtration can be prevented in mammals by injecting acacia, even though the proteins are reduced. Fahr and Kerkhof (1933) produced hypoproteinemia by plasmapheresis but prevented edema from appearing by substituting gum acacia for the missing protein. Though edema is produced by lowering the protein content of the blood, it is the low colloid osmotic pressure of the blood and not the loss of protein as such that is responsible for the edema.

The addition of plasma protein in the form of blood transfusions can aid in producing diuresis and subsidence of the edema accompanying nutritional hypoproteinemia (Landis and Leopold, 1930), though it is usually of no value in nephrosis due to the copious albuminuria characteristic of the latter condition. Hartman and Senn (1932) raised the colloid osmotic pressure of the blood in nephrosis by intravenous injection of gum acacia and observed subsequent diuresis and subsidence of edema. Weech, Goettsch and Reeves (1933) failed to obtain a similar effect with serum transfusions in edematous dogs, the amount of serum transfused being, however, too small to raise plasma albumin concentration to anything approximating normal value. Hyperproteinemia diminishes for a time the total amount of dye leaving the circulation (Smith and Dick, 1932) but this is ascribed to binding of dye by protein.

The observations so far cited provide qualitative evidence that the plasma proteins are concerned with the movement of fluid through the capillary wall under abnormal conditions. Krogh, Landis and Turner (1932) studied quantitatively in normal subjects the effect on fluid movement of changing the colloid osmotic pressure of the blood. Quiet standing increases the amount of protein in the circulating blood since protein-poor fluid is filtered from the blood into the dependent tissues.

By comparing the filtration produced in the forearm by a given venous pressure while the subject stood and reclined it was possible to determine the effects which measured changes in colloid osmotic pressure exert on filtration rate. A unit rise (1 cm. water) of venous pressure increased filtration by average values between 0.0023 and 0.0033 cc. per minute per 100 cc. of forearm. In six experiments a unit rise (1 cm. water) of colloid osmotic pressure diminished filtration by 0.0027 to 0.0045 cc. per minute per 100 cc. of forearm.

*Summary.* The available evidence, direct and indirect, indicates that the colloid osmotic pressure of the blood retains fluid within the vascular system and acts quantitatively in opposition to capillary pressure. In accord with physical principles the effects of capillary pressure and colloid osmotic pressure are opposite in sign but have the same order of magnitude.

3. *Impermeability of the capillary wall to plasma proteins.* To use the term "permeability" in accordance with the definition given on page 404 requires under the simplest possible conditions information concerning at least four quantities,—mass, area, time and pressure. When the permeability of the capillary wall is studied many additional factors affect the movement of substances. So complicated are the forces involved that it is doubtful whether use of the term "capillary permeability" has ever been based on completely adequate information. Thus alterations in the vitality of the capillary wall, internal and external hydrostatic or osmotic pressures, changing area, reabsorption, and staining affinities have each, at one time or another, obscured a complete understanding of the true properties of the endothelium itself.

It is generally agreed that crystalloids pass through the capillary walls with ease and, with greater or less rapidity, reach an equal concentration inside and outside the endothelial barrier separating blood and tissue fluid. When concentration is thus equalized crystalloids can no longer influence the movement of fluid through the capillary wall, though they do affect fluid movement temporarily before this equalization of concentration is reached. Under conditions of equilibrium, however, the transfer of fluids through the endothelium will depend in part upon the colloids inside and outside the capillary. The colloid osmotic pressure of blood when measured *in vitro*, is determined with an artificial membrane wholly impermeable to proteins. To apply these measurements to biological problems requires specific information concerning the permeability of the capillary wall to the plasma proteins.

During inflammation the endothelium permits leucocytes to pass;

yet potential fissures, if present in the capillary wall, play no part in the filtration of fluid or dyes under ordinary circumstances. Krogh (1929) describes the retention of dialyzed and filtered India ink within capillaries while plasma is completely filtered off after the application of urethane. Ink particles are retained also after chemical and mechanical injury of the endothelium (Landis, 1927a), indicating that even the damaged capillary wall can hold back particles having an average diameter of 200 millimicra. Florey (1926a) perfused the intestinal blood vessels of the dog with potassium ferrocyanide and iron ammonium citrate, fixing the tissue immediately afterward by perfusing with formalin and HCl. Prussian blue was distributed equally through the endothelial cells except for the nuclei. During inflammation the perfusion of starch and fixation with formalin and iodine showed starch within the cytoplasm of the endothelial cells, but the deposit was more copious on the inner side. Both substances passed into and through the capillary wall diffusely, without evidence of microscopically visible fissures or pores. Whatever may be the mechanism by which leucocytes pass through the capillary wall, it appears in the light of present information to have no direct relation to the normal filtration of fluid and dissolved substances.

The permeability of the capillary wall to protein could be defined easily and exactly if a true capillary filtrate were obtainable. The three fluids which can be distinguished are *a*, blood plasma within the capillary; *b*, tissue fluid bathing the external surface of the capillary, and *c*, lymph collected from small or large lymphatic ducts. Normal tissue fluid exists in very small amounts and consists probably of the original capillary filtrate mixed with capillary filtrate which has been modified by partial reabsorption. Lymph consists of tissue fluid which, after bathing the cells of the tissue, passes through the lymphatic endothelium and usually through lymph glands, all involving possible modifications in composition (Starling, 1909). The inaccessibility of the essential capillary filtrate and the minute size of the capillaries require that information concerning endothelial permeability be gathered primarily by indirect methods. Tissue fluid offers the nearest approach to capillary filtrate but reabsorption often plays a part in determining its composition and the amount of tissue fluid is normally so small that its analysis has so far not been accomplished. Endothelial permeability to protein has been perforce studied indirectly by analysis of *a*, edema fluid; *b*, blood collected under varying conditions, and *c*, lymph.

The concept of fluid balance proposed by Starling (1896) and elaborated

by Schade (1927) was used by Drinker and Field (1931) to explain the relation between capillary filtrate, tissue fluid and lymph. To use the words of the last-mentioned authors, a possible mechanism of lymph formation is . . . "that the filtrate from the blood capillaries to the tissue spaces contains water, salts and sugar in the concentrations found in the blood, together with serum globulin, serum albumin and fibrinogen in low concentration, lower probably than that of tissue fluid and lymph; that water and salts are reabsorbed by the blood vessels and the protein enters the lymphatics together with water and salts in the concentration existing in the tissue fluid at the moment of lymphatic entrance." Except for protein and the substances combined with protein (calcium and total phosphorus) the concentrations of the constituents investigated are nearly the same in plasma and subcutaneous lymph (Heim, 1933). Lymph also reflects very rapidly changes in the diffusible constituents of serum (Arnold and Mendel, 1927; Heim and Berg, 1933). The difference between normal tissue fluid and true capillary filtrate is due therefore primarily to reabsorption of water and crystalloids with simultaneous concentration of the protein in the extravascular fluid. The analysis of extravascular fluid, which has accumulated when reabsorption is completely prevented, should provide the most accurate estimate of the amount of protein leaking through the capillary wall.

In advanced nephrosis and malnutrition the colloid osmotic pressure of the blood is apt to be lower than capillary pressure. Reabsorption by purely physical forces is then impossible and the edema fluid thus produced should, in the absence of secretory activity on the part of the capillary wall, resemble the original capillary filtrate. The proteins of edema fluid are physically similar to those of blood plasma (Cavett and Gibson, 1931). If the protein content of edema fluid is expressed in terms of percentage of plasma protein the efficiency of the capillary wall in retaining plasma protein can be estimated. The objection may be raised that in edema the vascular endothelium is abnormal, but capillary injury has thus far been found always to be associated with increased capillary permeability. The protein content of the edema fluid would then be, if anything, greater than that of normal capillary filtrate. It is also true that the plasma proteins are reduced in nephrosis and malnutrition so that less protein would be expected in the edema fluid, but this error can be avoided by comparing the protein content of edema fluid with the corresponding plasma protein.

Beckmann (1921) found that subcutaneous edema fluid, in cases of "tubular nephritis" and amyloid kidney, contains under 0.1 per cent of

protein. Vančura (1931) observed in nephrosis 0.14 to 0.49 per cent protein. The colloid osmotic pressure of certain edema fluids is too small to measure accurately (Schade and Claussen, 1924). Landis and Leopold (1930) examined the ascitic fluid in a case of malnutrition and found 0.04 per cent protein while Meyer and Friedheim (1931) found in nephrosis from 0.15 to 0.27 per cent protein. The protein in peritoneal fluid remains low while fluid is moving into and out of the blood stream (Schechter, 1931). Landis et al. (1932) stated that the subcutaneous edema fluid of a nephrotic patient contained 0.09 per cent protein with a plasma protein of 3.7 per cent, indicating that the capillary wall retained over 95 per cent of the blood protein.

In chronic plasmapheresis also the edema fluid contains less than 0.25 per cent protein and at times less than 0.1 per cent (Leiter, 1928; Shelburne and Egloff, 1931). In another series of experiments ascitic fluid contained between 0.10 and 0.75 per cent protein with an average of 0.36 compared to an average serum protein of 5.88 per cent,—showing nearly 95 per cent retention (Greene et al., 1931).

Venous congestion also reduces to a minimum the absorption of fluid from the capillary filtrate since capillary pressure is raised above the colloid osmotic pressure of the plasma proteins. According to Beckmann (1921) edema fluid removed from patients with cardiac decompensation contains from 0.1 to 1.0 per cent protein with an average of 0.4 per cent. Landis et al. (1932) found edema fluid produced by local venous obstruction contained 0.29 per cent protein with a plasma protein of 6.64,—nearly 95 per cent of the blood protein being retained.

Inflammatory conditions and, to a less extent, cardiac decompensation generally produce edema fluids with a higher content of protein, the subcutaneous edema fluid containing in general less than collections in the serous cavities. Damage of the capillary wall is held to be responsible for the high protein content of inflammatory exudates. It is likely, therefore, that the lowest figures obtainable in studies of transudates (during their formation and before reabsorption begins) represent most nearly the protein content of normal capillary filtrate. At least these studies of edema fluid indicate that the capillary wall can, and in the absence of injury often does, retain approximately 95 per cent of the plasma protein.

Capillary permeability to protein has been estimated also by observing modifications in the concentrations of erythrocytes and blood proteins under various conditions. Such studies are made difficult, however, by the constantly varying diameter, pressures, and flow in the vessels which form the capillary network. To reduce the number of variables Landis

(1927b) studied, by a micro-injection method, the relation which capillary blood pressure bears to the direction and rate of fluid movement through the walls of single capillaries in the frog's mesentery. In this tissue, when capillary pressure exceeds 12.0 cm. of water, filtration occurs, the rate of filtration being proportional to the difference between capillary pressure and 11.5 cm. of water. When capillary pressure is below 9.0 cm. absorption occurs. Since neither absorption nor filtration occur with capillary pressures in the vicinity of 11.5 cm. of water, this figure represents the effective colloid osmotic pressure of frog's blood in the mesenteric capillaries. This value, determined *in vivo*, agreed approximately with the colloid osmotic pressure of frog's plasma measured *in vitro* by White (1924a). Much lower (and occasionally much higher) colloid osmotic pressures have been reported for frog's plasma by Krogh (1922) and by Churchill, Nakazawa and Drinker (1927) explaining (vid. inf.) the variable and often high rates of lymph formation in this animal.

According to Yamaguchi (1927a) the increase in plasma protein relative to erythrocytes during venous congestion in dogs and man indicates that the capillary wall of mammals is only slightly permeable to protein. The fluid lost from the blood stream of men during venous congestion produced by quiet standing was stated to be practically protein-free by Thompson, Thompson and Dailey (1927), while Waterfield (1931a) concluded that only globulin was held back, the albumin leaking through the capillary wall. Landis, et al. (1932) observed in human subjects that at very high venous pressures (80 mm. mercury or more) considerable protein passes through the walls of the capillaries in the forearm, though at lower venous pressures the capillary wall remains relatively impermeable to protein. This last observation and the use of different methods of determining blood volume may explain the discrepancy in findings during quiet standing.

If large amounts of protein were lost through the normal capillary wall it would be expected that raising colloid osmotic pressure by a given amount should affect the rate of filtration very much less than changing venous pressure by a similar amount. In observations on man Krogh, Landis and Turner (1932) found it difficult to associate the quantitatively similar effects of changing venous pressure and colloid osmotic pressure with any considerable leakage of proteins through the human capillary wall. Landis, Jonas, Angevine and Erb (1932) elevated venous pressure to 20, 40, 60 and 80 mm. of mercury and calculated from hematocrit determinations and analyses of plasma protein the amount of fluid and

protein lost during filtration. At venous pressures up to 60 mm. of mercury the amount of protein in the capillary filtrate was calculated to range from 0.0 to 0.7 per cent with an average of 0.3 per cent. It was concluded that during mild venous congestion the capillary wall retains approximately 95 per cent of the blood protein.

Analyses of lymph represent a third source of information concerning capillary permeability to protein. The mechanism of lymph formation has been reviewed recently by Drinker and Field (1933). Since lymph represents the original capillary filtrate modified by more or less reabsorption of fluid, caution must be used in estimating from analyses of lymph the constitution of the original capillary filtrate and therefore the true permeability of the capillary wall to protein.

Starling (1894), on the basis of analyses of lymph in dogs, stressed the differences in the permeability to protein exhibited by the capillaries of the limbs, intestine and liver. Regional differences exist also in the capillaries of the frog. Freund (1922) found the capillaries of the frog's hind limb, including chiefly muscle, are impermeable to protein under normal conditions though they become permeable after the injection of arsenic. According to Pak (1926) the capillaries of the hind leg of the frog retain Congo red and permit ammonium chloride, urea, glucose and certain alkaloids to pass with great ease. While micro-injection measurements of filtration and absorption (Landis, 1927b) indicated that the capillaries of the frog's mesentery are practically impermeable to protein, Churchill, Nakazawa and Drinker (1927) found simultaneously that subcutaneous lymph in the frog contains normally from 0.29 to 2.17 per cent of protein derived from leakage of plasma protein through the permeable cutaneous capillaries. This cutaneous lymph has an average osmotic pressure of 4.2 cm. water compared to 7.1 cm. for the blood serum. Since the average cutaneous capillary pressure is between 10 and 14 cm. water (Landis, 1931), fluid must be filtered throughout the entire capillary network of the skin. Lymph should therefore be produced in large amounts and, in the absence of reabsorption, its protein content measures directly the permeability of the cutaneous capillaries to protein.

Isayama (1924a, b) dissected out the lymph hearts of frogs and estimated, from temporary changes in the erythrocyte count of the blood, that in twenty-four hours a volume of fluid equivalent to 50 times the blood volume may pass out of the blood capillaries. Essentially similar findings were reported by Ito (1926). Conklin (1930a) collected lymph from the crural sacs and found that fluid leaves the blood at rates cor-

responding to the filtration of seven to forty times the calculated blood volume in twenty-four hours. Curarization, which stops the lymph hearts, produces edema and elevates body weight by 10 to 32 per cent in six hours. When edema is produced by injecting Ringer's fluid into the circulation the skin absorbs most fluid,—indicating, like the studies of lymph, that the cutaneous capillaries of the frog retain less fluid than capillaries elsewhere. The combination of highly permeable cutaneous capillaries with lymph hearts and rapid circulation of body fluids represents, therefore, a specialized mechanism, which must be kept in mind in studies of fluid balance in amphibians.

The easy passage of proteins through the cutaneous capillary walls of the frog was demonstrated by Conklin (1930b). Intravenous injection of large quantities of Ringer's fluid produces a copious flow of protein-containing lymph and the blood protein may be reduced eventually to very low levels. Hemoglobin and hemocyanin pass easily, though egg albumin and gelatin pass with difficulty and then only after the endothelium has been rendered abnormally permeable by the injection of Ringer's fluid. These findings amply verify the statement of Drinker (1927) that the capillaries of the frog's foot may "leak protein-containing fluid at all diameters."

With such rapid movement of fluid from blood, through the capillary wall, into the lymph spaces it is entirely justifiable to assume that capillary filtrate, tissue fluid and lymph are practically identical under normal conditions (Drinker, 1927) since there is little or no opportunity for reabsorption of fluid from the original capillary filtrate. But this relation between capillary filtrate and lymph may well be limited to certain animals and tissues. If available data be accepted the same condition does not exist in the mesentery of the frog nor in the limbs of mammals, where protein is retained more completely and where reabsorption takes place.

Drinker and Field (1931) called attention to the high and variable concentration of protein in mammalian lymph, concluding that this protein must have passed through the capillary wall in order to reach the lymphatics. The presence of blood protein in lymph makes it unlikely that the capillary endothelium is totally impermeable to the plasma proteins, but it does not rule out the possibility that the original leakage of protein is extremely slight. It is also conceivable, though far from likely, that the tissue cells or lymphatics fabricate proteins which find their way into lymph.

The importance of the colloid osmotic pressure of the blood in deter-

mining the rate and direction of fluid movement depends upon the degree to which protein is retained by the capillary endothelium. If the capillary filtrate contains very small amounts of protein compared to the blood plasma the effective colloid osmotic pressure of the blood will be equal to the colloid osmotic pressure measured *in vitro*. On the other hand, if large amounts of protein are lost in the capillary filtrate the concept of a balance between capillary pressure and the colloid osmotic pressure of the blood becomes untenable.

The passage of protein through the capillary wall in immunologically detectable amounts has been demonstrated under normal and experimental conditions. Field and Drinker (1931a) injected sterile horse serum intravenously and demonstrated its presence in the cervical lymph within one to two hours by a precipitin reaction. Serum injected subcutaneously found its way rapidly into the lymphatics. The protein passes slowly through the capillary wall, and once outside the capillary it enters the lymphatics almost at once, the lymphatic endothelium being presumably freely permeable to protein. When horse serum is injected subcutaneously no absorption of foreign protein through the capillary wall is detected during periods up to an hour. This indicates either that the capillaries of the subcutaneous tissues are permeable to protein in one direction only or that extraneous physical forces deter protein from entering the blood stream. After plasmapheresis, however, when the plasma protein is reduced, and blood pressure lowered, foreign protein placed in the subcutaneous tissues can be detected serologically in the blood though entrance *via* lymphatics is avoided (Field and Drinker, 1931b). Hence Field and Drinker conclude that the capillary wall permits protein to pass in either direction as an inert membrane should, providing extraneous physical factors do not prevent such passage. This passage of protein takes place under exceptional circumstances; under normal conditions the capillary wall is not concerned with the removal of protein from the tissue spaces. Selective removal of protein from the tissue spaces by lymphatic endothelium has been suggested (Peters, 1933) but for this no direct evidence is available at present.

Qualitative passage of protein through the capillary wall into the lymph having been demonstrated, the amount of leakage of protein must be considered because of its relation to the effective colloid osmotic pressure of the blood. In considering the high and variable protein content of mammalian lymph Drinker and Field (1931) suggested that tissue fluid and lymph are identical on account of the ease with which large molecules and even particles enter the lymphatics. Motion or

massage causes the lymphatic wall to lose its anatomical integrity and to become continuous with the tissue spaces.

Churchill, Nakazawa and Drinker (1927) point out that in the frog the effective colloid osmotic pressure for restoring fluid to the capillary blood is the difference between the colloid osmotic pressure of the blood and that of subcutaneous lymph. This conclusion is justifiable for the cutaneous capillaries of the frog where fluid moves from blood to lymph channels rapidly with little or no reabsorption (Isayama, 1924; Ito, 1926; and Conklin, 1930a). This concept was applied by Loewen, Field and Drinker (1931) to the dog. The colloid osmotic pressure of the blood amounted to 37.7 cm. of water compared to 16.6 cm. water for cervical lymph. It was held that the effective colloid osmotic pressure for returning water to the blood is the difference between the colloid osmotic pressure of the blood and the lymph from the area under observation. This difference amounts to 21.1 cm. water, considerably less than 35–37 cm. water,—the colloid osmotic pressure of blood when measured *in vitro*. However, reabsorption is not absent nor is lymph commonly produced in mammals at a rate comparable to its flow in the frog. Assuming the correctness of the direct capillary pressure determinations in mammals, reabsorption by purely physical means would be almost impossible and fluid should be filtered constantly and rapidly from the cervical capillaries of the dog, whereas massage is usually required to obtain any lymph during rest.

If the effective colloid osmotic pressure of the blood were merely the difference between the colloid osmotic pressures of blood and lymph a paradoxical situation would result. During rest lymph flows slowly and contains much protein, a low effective colloid osmotic pressure being associated with marked reabsorption of fluid. During muscular activity it is generally agreed that lymph flows more rapidly and contains less protein, a high effective colloid osmotic pressure being associated with less absorption of fluid. The true effective colloid osmotic pressure of the blood is the difference between the colloid osmotic pressures of the capillary filtrate and blood plasma. This should be a fairly constant figure for any one tissue and animal except when injury of the endothelium permits large amounts of protein to pass. It was in this sense that the term "effective colloid osmotic pressure" was used in studies of fluid movement during inflammation and injury of the frog's mesenteric capillaries (Landis, 1927b).

Krogh, Landis and Turner (1932) found that no filtration of fluid could be detected in the human forearm until venous pressure was

elevated to 15 cm. water. They found it difficult to associate this so-called margin of safety with the very low effective colloid osmotic pressure that must result from the general presence of an extravascular fluid containing from one-third to one-half as much protein as blood. From the filtration-absorption concept adopted by Starling (1896), Schade (1927), and Drinker and Field (1931) it follows that tissue fluid must vary from a true capillary filtrate with low protein content just outside the filtering capillary areas to a concentrated fluid containing more protein near the absorbing areas. Lymph, being composed partly of the latter type of tissue fluid, must usually contain more protein than true capillary filtrate. It is doubtful whether analyses of normal lymph can in general provide definite information concerning the average protein content of the tissue fluid surrounding the capillary wall and particularly concerning the concentration of protein in the original capillary filtrate. The protein in lymph must vary widely depending, among other things, on the rate of filtration and the relative amount of absorption. The slower the filtration and the more complete the reabsorption, the more will the composition of lymph differ from that of the capillary filtrate and from that of average tissue fluid. The more rapid the filtration and the less complete the reabsorption, the more closely will both tissue fluid and lymph resemble the fluid filtered through the capillary wall.

In order to determine from analyses of lymph the protein content of the original capillary filtrate, it is necessary to collect lymph *a*, without reabsorption, and *b*, without injury of the capillary wall. Both of these factors, if present, will tend to produce extravascular fluid which contains more protein than does the filtrate passing originally through the normal capillary wall. Results obtained from studies of lymph can be brought into general agreement with those obtained from analyses of edema fluid and blood, when allowance is thus made for the high protein concentration sometimes, but not always, observed in lymph. The permeability of the lymphatic endothelium must also be remembered for its possible importance in determining the composition of lymph (McMaster and Hudack, 1932a, b; Peters, 1933).

The composition of subcutaneous lymph has been studied recently during sterile inflammation, extreme venous congestion, plasmapheresis (Field and Drinker, 1931c), muscular activity, rest and mild venous congestion (White, Field and Drinker, 1933).

Immersing the leg of a dog in hot water increases the flow of lymph and the protein content of the lymph rises to 4 per cent or more. This leakage of protein is obviously due to capillary injury. Ligation of all

the leg veins increases the rate of lymph flow and the protein in lymph simultaneously decreases, but 1.02 per cent is the lowest figure reported. Such complete venous ligation not only raises venous pressure to extremely high levels, but stops blood flow. Reabsorption is impossible, but anoxemia makes the capillary wall abnormally permeable to protein (Landis, 1928). Marked venous congestion of the human forearm also produces a capillary filtrate containing considerable protein (Landis et al., 1932).

Acute plasmapheresis increases the flow of cervical lymph and lowers its protein concentration to between 0.86 and 1.25 per cent (Field and Drinker, 1931c), the lymph protein percentage tending to follow variations in plasma protein percentage. Lymph collected after acute plasmapheresis contains considerably more protein than does edema fluid removed during chronic plasmapheresis. As Field and Drinker (1931c) mention, reabsorption is eliminated and the lymph flows so rapidly that it must resemble the original capillary filtrate quite closely. The higher protein content may, however, be due to capillary damage with increased permeability owing to rapid protein depletion. Whipple, Smith and Belt (1920) describe a shock-like state following rapid plasmapheresis. Death was produced within 1.5 hours and was ascribed to tissue injury. Conklin (1930b) found also that extensive flushing with Ringer's fluid rendered the vascular endothelium of the frog more permeable to colloidal substances. Acute plasmapheresis is accomplished by a few large injections of Ringer's fluid or salt solution in a relatively short time, while in chronic plasmapheresis numerous smaller injections are made over a period of days. This may be responsible for the greater protein leakage in the acute experiment.

During walking the lymph from the feet of dogs contains on the average 1.0 per cent of protein, but 0.5 per cent is not uncommon (White, Field and Drinker, 1933). It is impossible to state that reabsorption has been entirely avoided, nor is there any definite knowledge, as Drinker and Field (1933) mention, of the exact line of demarcation between normal and abnormal capillary permeability. It is conceivable that even in normal muscular activity temporary asphyxia or compression of turgid capillaries by contracting muscle fibers might modify capillary permeability so that more protein leaks through the endothelium than would pass through the same region during rest.

In man (Drinker and Field, 1933) subcutaneous lymph obtained during rest contained 0.69 per cent protein while walking reduced it to 0.49 per cent. Lymph obtained by massage of resting tissues may contain up to

4.5 per cent protein, this higher content being explicable on the basis of reabsorption.

Elevating venous pressure slightly by inflating a pneumatic cuff at the base of the dog's leg increases lymph flow and diminishes the protein concentration. The lymph contained in one observation 0.21 per cent protein and in another 0.78 per cent (White, Field and Drinker, 1933). Thus when capillary pressure is elevated slightly, with little or no local asphyxia, the protein content of lymph may be extremely low,—much lower than that observed ordinarily during rest or even during exercise. Such moderate elevation of capillary pressure limits reabsorption, but still is not so likely to produce anoxemia. Under such conditions lymph would be most apt to resemble capillary filtrate and would most nearly reflect the permeability of the normal capillary wall. These findings compare favorably with the very slight protein loss described for human capillaries by Landis et al., (1932) when venous pressure is raised but slightly.

It seems very likely that reabsorption and endothelial injury explain why lymph contains under certain conditions only 0.21 per cent protein and under other conditions over 5.0 per cent. If this reasoning is correct the available studies on lymph agree with the studies on edema fluid and blood in showing that the capillary endothelium can, and often does, retain 95 per cent of the plasma protein. The effective colloid osmotic pressure is then very slightly less than the colloid osmotic pressure of the blood measured *in vitro*.

The fact that small amounts of protein are found in edema fluid and in lymph obtained during slight venous congestion invites inquiry into the manner in which protein leaks through the capillary wall. Protein might pass in very low concentration through the entire endothelial surface or unmodified plasma might leak through a few capillaries which are abnormally permeable owing to trauma, or to a natural process of ageing. If the first possibility were correct lymph would probably contain more albumin than globulin owing to the smaller molecular weight of albumin. If the latter condition existed the albumin-globulin ratio of lymph would tend to resemble that of blood. Loewen, Field and Drinker (1931) found that the specific colloid osmotic pressure of subcutaneous lymph per gram of protein is higher than that of plasma and suggested that this might be due to a greater proportion of serum albumin in lymph. Wells (1932) on the other hand found in nine experiments that the albumin-globulin ratios of serum and of lymph from the mesenteric lacteal vessels of dogs are approximately similar. The ratios agreed

satisfactorily with direct estimations of colloid osmotic pressure. The capillary filtrate produced in the human forearm by marked venous congestion contains both albumin and globulin (Landis et al.,1932). Weech, Goettsch and Reeves (1933) also found that the albumin-globulin ratios of serum and subcutaneous lymph are mutually dependent variables. Pathological collections of fluid, on the contrary, frequently show a higher specific colloid osmotic pressure than blood indicating a preponderance of albumin (Peters and Van Slyke, 1931; Meyer, 1932). Nothing final can be said concerning the manner in which protein passes through the normal and injured capillary wall. If the albumin-globulin ratios of lymph are significant, proteins may, under normal conditions, leak through relatively few pores large enough to permit both albumin and globulin to pass in the ratio existing in blood. Pathological increase in capillary permeability, however, may be accompanied by leakage of more albumin than globulin.

The loss of fluid from the blood stream which accompanies quiet standing increases not only the concentration of blood protein but also the concentrations of cholesterol, phosphatides, and compounds of saturated and slightly unsaturated fatty acids, showing that not only protein but also other substances are retained by the capillary wall (Man and Peters, 1933). Though substances other than protein affect the colloid osmotic pressure of the blood, their significance in this respect is limited (Kylin and v. Pein, 1931; Fishberg, 1929; Macheboeuf and Sandor, 1931; Meyer, 1932; Achard, Grigaut and Codounis, 1930).

*Summary.* The physical factors involved in the movement of fluid and dissolved substances through the vascular endothelium are so complex that changes in capillary permeability should be described only after careful control of the forces known to influence the movement of substances through an inert membrane. Ever varying capillary pressure and rate of blood flow in the units of the capillary network add further complexity. Analyses of edema fluid, blood and lymph indicate that the capillary wall is usually relatively impermeable to protein. In many tissues the endothelium normally retains at least 95 per cent of the total plasma protein. Generalizations should not be made to apply to all capillaries since regional differences in permeability exist. Lymph resembles the original capillary filtrate most closely when reabsorption and capillary injury have been completely avoided. The leakage of protein through the capillary wall in many tissues is so slight that it is justifiable to assume that normally the effective colloid osmotic pressure of the plasma proteins in the capillary network is practically the same as the

colloid osmotic pressure of the plasma when measured *in vitro* with a membrane which is totally impermeable to protein.

4. *Diffusion and the permeability of the capillary wall to electrolytes.* The exchange of highly diffusible solutes to which the capillary wall is permeable need not follow the current of water during either filtration or absorption. When hypertonic solutions of electrolytes are introduced intravenously the electrolyte diffuses through the capillary wall toward the tissue spaces even while fluid is moving toward the blood. This is fortunate since (Peters, 1933) if diffusion of solutes followed the current of solvent only, the establishment of electrolyte and osmotic equilibrium among body fluids would be much slower than it is. Thus after glucose solutions are introduced intraperitoneally water and electrolytes pass into the injected fluid while glucose diffuses into the blood simultaneously (Clark, 1921; Schechter, 1931). On account of the time required for the diffusion of electrolytes, fluid movement through the capillary wall is modified temporarily (White and Erlanger, 1920; Weed and McKibben, 1919; Kubie, 1928; Kinsman, Spurling and Jelsma, 1928, and others) but the effect is transient, disappearing when the concentration of electrolytes is once more equal inside and outside the capillary wall. The time required to restore equilibrium depends upon the rapidity with which the substance diffuses through the capillary wall. Diffusion is doubtless aided by a current of water moving in the same direction. Whether diffusion can be hindered appreciably by fluid moving through the capillary wall in the opposite direction is not known though from rough calculation it seems unlikely.

The independence of fluid movement and the diffusion of electrolytes is perhaps best illustrated by the behavior of certain dyes which have been used extensively to investigate the resistance which the capillary wall offers to the passage of substances whose molecular size lies between those of plasma protein and crystalloids. The passage of dyes can be followed easily since they are usually visible in low concentration. Interpretation of results, on the other hand, is often difficult owing to complicating factors such as electrical charge, selective staining, adsorption, toxicity, decolorization or deposition in the tissues. Dyes are, moreover, foreign substances and many are collected selectively by cells whose function it is to rid the body of extraneous materials.

Schulemann (1917) showed that those acid dyes which diffuse slowly into gelatin do not show, after subcutaneous injection, any great degree of general vital staining while those diffusing rapidly show conspicuous vital staining. He concluded that dyes pass into the blood stream

from the subcutaneous tissues by diffusion and that their relative diffusibility through the capillary endothelium is the same as that through gelatin.

The rate at which dye solutions pass through the capillary wall has been referred to various chemical and physical properties of the dyes used (Wittgenstein and Krebs, 1926; Shimidzu, 1922; Pohle, 1924). With any single suitable dye, however, the state of the capillary wall itself is usually taken to be the chief factor in determining the rate of passage. Rogowicz (1885) observed that a dye introduced into the saphenous vein passes into the tissues more rapidly whenever active hyperemia is induced by stimulation of vasodilator nerves or by division of vasoconstrictor fibers. Krogh (1922) states that two relatively indiffusible dyes, brilliant vital red and Chicago blue VI B, pass very slowly through the normal capillary wall, but with the production of hyperemia or stasis the vessels are quickly surrounded by a deeply stained zone. Likewise the active hyperemia resulting from heating of the skin (Okuneff, 1924) permits in the affected area a more conspicuous staining of the tissues after intravenous injection of trypan-blue. Anitschkov (1924) attributed this effect to local dilatation of the capillaries, with increased permeability due to stretching, a suggestion originally made by Krogh (1922). Dyes follow the current of fluid under these conditions.

When dye solutions are injected through micro-pipettes into single capillaries of the frog's mesentery, vessels of like diameter, even though they arise from the same arteriole, permit pigment to appear outside the endothelium after periods of time which differ widely in various capillaries (Landis, 1927b). These variations in the rate of passage of dye solutions depend primarily upon the level of capillary pressure existing in each vessel and very little, if at all, upon the diameter of the capillary. At a given pressure solutions of various dyes must be perfused through single capillaries for different periods of time before the pigment appears outside the endothelium. This was related to differences in the colloidal properties of the dyes used (Landis, 1927b).

Vital red HR injected in relatively small quantity into the blood stream of the frog leaves some mesenteric capillaries and fails to leave other capillaries in the same region. In those capillaries where no dye passes the pressure is generally below 11 cm. of water, while in those where dye passes rapidly the capillary pressure is almost always over 14 cm. of water. Diverting the flow of an arteriole into a single capillary makes dye pass quickly through the endothelium, though under normal

pressure conditions none has passed. It was also noted that after some delay considerable dye leaves the smaller venous capillaries and the smallest venules, so that finally the connective tissue surrounding these vessels is deeply stained. This passage of dye from venules was ascribed by Landis (1927b) to selective "vital staining" of the connective tissue. The relation between capillary pressure and the passage of dye-stained fluid from capillaries was taken to be corroborative evidence that capillary pressure influences the movement of fluid through the endothelium. In such cases diffusion of dye is aided by a current of fluid passing in the same direction.

Certain dyes pass through the walls of veins before any visible passage through the capillary endothelium can be detected (Frölich and Zak, 1924; Runge, 1927). Rous, Gilding and Smith (1930) injected acid vital dyes intravenously and studied the staining of tissues about the capillaries and veins. Highly diffusible dyes escape so rapidly from the capillaries of mammalian muscle that the tissue surrounding the arterial end of the capillary is colored even before the venous end is reached by the dye-stained blood. Less diffusible dyes emerge all along the muscle capillaries, but in greater amount toward the venous end. Poorly diffusible dyes at first fail to appear outside the arteriolar portion of the capillary network but escape in increasing amount in the venous portion. Eventually in all cases the tissue becomes colored evenly. The greater accumulation of poorly diffusible dyes in the vicinity of the venous capillaries and venules is but little affected by the changes of circulation and fluid movement which accompany muscular activity (except tetanus), plethora, severe hemorrhage, nerve section, shock, dehydration and injections of epinephrin and pituitrin.

Capillary pressure and fluid movement do not especially favor the passage of dye through the venous capillaries, since in this region blood pressure is lower and filtration much less likely than in the arteriolar capillaries. Moreover, dyes pass through the endothelium even when intravascular pressure is reduced nearly to zero. Rous, Gilding and Smith (1930) developed the hypothesis that the endothelium of the capillaries serving certain organs is relatively impermeable near the arterioles and becomes increasingly permeable as the venules and veins are approached. Anatomical and physiological reasons were advanced to indicate that this gradient of capillary permeability ensures equal distribution of diffusible substances to the tissues. They pointed out that the absence of a demonstrable gradient of permeability in the liver and bladder of mammals and in the muscles of birds is associated with an

anatomical arrangement of capillaries which in itself ensures such a uniform supply of the diffusible constituents of the blood (Smith and Rous, 1931a).

Under normal circulatory conditions, the gradient can be demonstrated only by means of moderately or poorly diffusible dyes; highly diffusible dyes like phenol red and patent blue V pass so rapidly through the entire endothelial surface that no difference in the rate of diffusion can be observed. Smith and Rous (1931a) state that the most highly diffusible material used, phenol red, spreads through water at the same rate as dextrose and through gelatin more than three times as slowly, lagging therefore far behind many substances used by the tissues, since the rate at which dextrose passes through the endothelium is slow compared to that of the salts. Patent blue V which, according to their tests, "most nearly approaches a normal foodstuff in diffusibility" passes out at once everywhere along the entire capillary wall and the field is obscured. Colored substances of simple constitution were either not sufficiently intense in hue for their escape from the vessels to be followed or else they proved toxic. The gradient is therefore demonstrable only with dyes whose diffusibility is much less than that of the blood constituents concerned in nutrition. To what extent observations with poorly diffusible dyes can justifiably be applied to the highly diffusible constituents of blood is still a matter of inference and conjecture.

Rous believes that the gradient of permeability is based upon anatomical differences along the capillary wall because local differences in the escape of dyes are still demonstrable despite great alterations in the circulatory state. Complete denervation does not obliterate the gradient, though widening its scope. Hesselmann (1932), on the other hand, found in urethanized frogs that sympathectomy obliterates all trace of the gradient and believes it to be functional in origin depending on innervation.

Though the deeper staining about the venous capillaries occurs in spite of drastic variations in capillary pressure it is much influenced by capillary pressure (McMaster, Hudack and Rous, 1932). Nerve section induces hyperemia and increases blood flow. After an intravenous injection of dye, denervated muscles are equally and evenly stained at a time when the control muscles still show the barring of color characteristic of greater escape of dye in the venous portion of the capillary network. Prior to diffuse staining, barring occurs also in denervated muscles but the bars of color are deeper and broader with a light general staining which the control muscle lacks entirely. Thus a generalized

staining is finally superimposed upon the local, more intense staining about the venous end of the capillaries.

Elevating venous pressure increases the escape of dye through the capillary wall. When venous congestion has persisted for some days even the veins become more permeable than usual. It was concluded that the gradient of permeability exists independently of variations in hydrostatic pressure, though raising venous pressure extends the scope of the gradient and accentuates local differences in permeability. If venous pressure is raised in the ear of the mouse so high that capillary pressure approaches the arterial pressure, dye escapes from the greatly distended capillary network without a trace of the gradient observed under ordinary conditions.

Rous and Smith (1931) repeated in modified form the experiment of Landis (1927b) on the frog's mesentery, injecting larger quantities of vital red HR intravenously. Landis injected only enough dye to color the plasma definitely while Rous and Smith used amounts great enough to render the corpuscles invisible. The patchy appearance of dye outside certain arteriolar and venous capillaries, found by Landis to be associated with higher capillary pressure by direct measurement, was ascribed by Rous and Smith to injury, since such "escape of stain in rapid and disorderly fashion from isolated capillary segments" was more frequently noted after prolonged irrigation of the frog's mesentery. Capillary pressure was not measured, however, and a much larger amount of dye was injected by Rous and Smith. This may explain the fact that the escape of dye about the venous capillaries occurred simultaneously with its passage from the true capillaries in the studies of Rous and Smith, while delayed somewhat in those of Landis. The amount of dye injected clearly conditions the extent and the rate of staining (Smith and Rous, 1931a).

The extent of the visible gradient of permeability and the intensity of staining are increased by diminishing the plasma colloids and are decreased by raising the concentration of the plasma proteins. In the first instance generalized staining with poorly diffusible dyes occurs in addition to the usual evidence of a gradient. The influence of plasma protein on the escape of dye from the blood is ascribed to adsorption of dye by protein (Smith and Dick, 1932).

An increased escape of dyes everywhere along the capillary over and above the localized staining described above accompanies hyperemia (with probable increase in capillary pressure), venous congestion (McMaster, Hudack and Rous, 1932), and capillary pressures above cer-

tain levels (Landis, 1927b). This general escape is probably due to the passage of a dye-containing filtrate of plasma. The staining about the further portion of the capillaries occurs even during the intravenous injection of hypertonic glucose solution (Smith and Dick, 1932) which is known to withdraw fluid from the tissues. Rous, therefore, holds this localized staining to be independent of fluid movement and to be due to diffusion which might well occur against a current of fluid moving inward through the capillary wall if the results of Ingraham, Lombard and Visscher (1933) and of Schechter (1931) may be cited in this regard. However, the filtration of dye-stained fluid is often superimposed upon this pure diffusion (Landis, 1927b; McMaster, Hudack and Rous, 1932).

Evidence has already been cited to indicate that the gradient of permeability influences but little the distribution of dyes which are similar in their diffusibility to the crystalloidal constituents of blood plasma. Whether or not blood proteins escape most readily through the further portion of the capillary there is at present no way of telling. In any case the total amount of protein passing through the capillary wall is small (vid. sup.). Tests with methemoglobin, Congo red and protein coupled with azo dyes yielded only an equivocal coloration (Smith and Rous, 1931b).

The evidence given above concerning the impermeability of the capillary wall to protein indicates that in most tissues the plasma proteins pass through the vascular wall only in relatively small concentration under normal conditions. In order essentially to modify the balance between capillary pressure and the colloid osmotic pressure of the blood, permeability must be increased sufficiently to permit plasma protein to pass freely. Thus the gradient of permeability, which has so far been demonstrated only by dyes, can play only a small rôle in the relation which fluid movement bears to the balance between capillary pressure and the colloid osmotic pressure of the plasma proteins. Landis (1928) in micro-injection experiments in the frog's mesentery found that increasing the hydrogen ion concentration of the irrigating fluid increases slightly the rates of filtration and of absorption which accompany a given difference between hydrostatic and colloid osmotic pressure, but does not modify the effective colloid osmotic pressure of the plasma protein until injury supervenes, when the effective colloid osmotic pressure of the blood is reduced. Only with conspicuous injury can the absorption of fluid be abolished, but absorption is slightly hastened by minor injury which is insufficient to reduce the effective colloid osmotic

pressure of the plasma protein. The greater permeability to dyes, if not great enough to allow the proteins to pass, can merely facilitate reabsorption since, for a given difference between capillary pressure and the colloid osmotic pressure of the blood, more fluid will be reabsorbed in unit time. Free diffusion, on the other hand, will be facilitated by greater permeability however small.

Peters (1933) points out that Rous' objection to the acceptance of the Starling concept fails to take into account that Starling was dealing with the forces that determine the passage of protein-free filtrate as a whole, and which hold back protein, not with processes which determine the diffusion of single solutes to which the vessel walls are permeable. He cites the experiments of Smith and Dick (1932) who showed that modifying water balance by changing the protein and electrolyte content of serum influences but little the distribution of dyes. According to Peters (1933) this at once establishes clearly the irrelevancy of Rous' experiments to water exchange and the Starling theory. Such gradients as Rous demonstrated "can at most equalize the distribution of nutrient materials of large molecular size as he has suggested but cannot appreciably influence the exchange of water or electrolytes."

*Summary.* The exchange of diffusible solutes, to which the capillary wall is more or less freely permeable, need not follow the current of water during either filtration or absorption. When the electrolyte equilibrium is disturbed, fluid movement is modified temporarily until diffusion equalizes the concentration and the osmotic pressure of the electrolytes inside and outside the capillary. The rates at which certain poorly diffusible dyes pass through the various portions of the capillary network indicate that the capillary wall becomes increasingly permeable to dyes toward its venous extremity. The greater passage of dyes through venous capillaries is independent of capillary pressure and fluid movement and is presumably due to pure diffusion. Yet elevating capillary pressure increases the passage of dyes everywhere along the capillary so that under appropriate conditions the filtration of dye-stained fluid may be superimposed upon pure diffusion of dye, and may even obliterate all evidence of local differences in endothelial permeability. This gradient of permeability tends therefore to equalize the distribution of poorly diffusible dyes but, in view of the available evidence, cannot appreciably modify the exchange of water or electrolytes.

5. *Tissue pressure and the movement of fluid through the capillary wall.* The pressure under which blood flows through the capillary network is but one point in the difference between the hydrostatic pressures inside

and outside the capillary wall. If the fluid filtered by an excess of capillary pressure over colloid osmotic pressure passed into a space which could expand easily without distention, a high venous pressure should produce filtration indefinitely at a constant rate. Observation shows, however, that this is not the case (Drury and Jones, 1927; Landis and Gibbon, 1933). In the tissues, and to a lesser extent in the serous cavities, the space available for accommodating extravascular fluid is limited; the tissue elements must be separated and further filtration of fluid is opposed by the pressure required to distort the tissues.

The external force required to depress the skin has been used to identify various stages of pre-edema and edema (Gildemeister and Hoffmann, 1922; Schade, 1926; Kunde, 1926; Meyer and Holland, 1932) but does not measure tissue pressure in absolute figures. Landerer (1884) was the first to estimate tissue pressure directly by introducing a fine needle or cannula into the cutaneous or subcutaneous tissues. He concluded that in rabbits tissue pressure is normally equivalent to about 5 to 7 cm. water with wide variations in different parts of the body and in different tissues. In man tissue pressures up to 55 cm. water were found, intracutaneous pressures being usually two to three times greater than the pressure in subcutaneous tissues. Venous congestion elevated tissue pressure conspicuously but the duration and the grade of venous congestion are not recorded.

Hajen (1927) measured the pressure required to form a cutaneous wheal, but this procedure separates the tissue elements far more suddenly and widely than does normal filtration. Drury and Jones (1927) observed that at a given venous pressure the rate of filtration, measured by a simple plethysmograph, is greater from the tenth to the twentieth minute of congestion than it is from the twentieth to the thirtieth minute. They were, however, unable to measure the volume of fluid filtered during the first ten minutes of congestion due to the limitations of the method used. According to Krogh, Landis and Turner (1932) an accumulation of fluid in the tissue spaces diminishes or even abolishes the filtration usually observed with venous pressures of fifteen to thirty centimeters water. The possible importance of lymphatic drainage and tissue pressure were both considered in the discussion of these results, but the limitations of the earlier pressure plethysmograph precluded any definite decision concerning the importance of tissue pressure. Later observations (Landis and Gibbon, 1933) with an improved plethysmograph indicated that tissue pressure is the more important. Filtration rates measured during the first few minutes of congestion are

uniformly higher than those observed during succeeding periods even when congestion continues during the entire period over which the accumulation of extravascular fluid is measured. Likewise the filtration rates observed in the forearm over ten and thirty minute periods of congestion are considerably higher than those reported for the leg by Drury and Jones who measured filtration only after venous congestion had been present for ten or more minutes. This discrepancy would be expected if a gradual increase of tissue pressure accompanied the accumulation of extravascular fluid.

According to Landis and Gibbon (1933) tissue pressure reduces filtration most rapidly during the first thirty minutes of venous congestion; at the end of this period the rate of filtration is often less than one-quarter that observed during the first five or ten minutes. When sufficient fluid has accumulated in the tissue spaces low venous pressures fail to produce further filtration. With higher venous pressures the filtration rate is decreased by an amount which is equivalent to a tissue pressure of 35 cm. of water. This figure was obtained indirectly and represents only the extent to which the effectiveness of a given capillary pressure is diminished when large amounts of extravascular fluid are present. In part this reduced rate of filtration is explained by the greater concentration of plasma protein owing to loss of fluid from the blood trapped in the congested vessels. The colloid osmotic pressure of blood removed from the veins of the foot during quiet standing is from 24 to 64 per cent greater than normal (Youmans, Wells, Donley and Miller, 1933).

A high tissue pressure increases also the rate at which extravascular fluid is removed from the tissue spaces (Krogh, Landis and Turner, 1932; Landis and Gibbon, 1933). It is impossible to state whether this fluid is removed *via* blood capillaries or lymphatics, though indirect results indicate (Krogh, Landis and Turner, 1932) that small accumulations are reabsorbed by the capillaries while larger accumulations are removed primarily through lymphatic channels.

In the human being tissue pressure helps to maintain normal blood volume against considerable hydrostatic disadvantage. Thus in the erect position, and particularly in quiet standing, when the veins are not emptied by muscular contraction, the capillary pressure in the dependent tissues is much higher than the colloid osmotic pressure of the blood. Yet normal individuals fail to show edema in the dependent tissues even though the standing position is assumed for relatively long periods of time. Filtration, though present, is reduced conspicuously long before

limb volume has increased by 10 per cent, which, according to Drury and Jones (1927), is the change required to produce pitting edema. The dangers of reducing blood volume too greatly are obvious. Quiet standing brings the vasular system very near to temporary failure and frequently produces fainting even of normal subjects (Turner, Newton and Haynes, 1930; Hamilton, Lichty and Pitts, 1932). Tissue pressure, by limiting the amount of fluid which is temporarily lost by filtration, reduces the need for cardiovascular readjustment during prolonged standing.

The earlier measurements of tissue pressure in man show little agreement, varying from 55 (Landerer, 1884) to 13 cm. water (Gildemeister and Hoffmann, 1922). According to more recent observations by Meyer and Holland (1932) normal cutaneous tissue pressure amounts to approximately 7 cm. water while subcutaneous tissue pressure is considerably less, ranging from 2 to 4 cm. water. While Landerer, and Gildemeister and Hoffmann both found tissue pressure increased during elevation of venous and capillary blood pressure, Meyer and Holland (1932) found no change when the extremity was placed below heart level. Using Landis' (1930b) figures for capillary pressure, Meyer and Holland (1932) have calculated that in man, when tissue pressure is allowed for, arteriolar capillary blood pressure exceeds the sum of the effective colloid osmotic pressure of the blood and tissue pressure by 50 cm. water. Venous capillary blood pressure, on the other hand, falls below the sum of the colloid osmotic pressure and tissue pressure by 220 mm. water. Krogh, Landis and Turner (1932) on different grounds suggested also that on the average in resting tissues the tendency toward reabsorption is greater than that toward filtration.

Equations have been used to clarify the manner in which capillary pressure, colloid osmotic pressure and tissue pressure influence fluid movement (Iversen and Johansen, 1929; Meyer and Holland, 1932; Weech, Snelling and Goettsch, 1933). At equilibrium with no fluid movement capillary blood pressure (CP) will be balanced by tissue pressure (TP) and the effective colloid osmotic pressure of the blood which consists of the total blood colloid osmotic pressure ($COP_B$) minus the colloid osmotic pressure of the extravascular fluid ($COP_T$).

$$CP - TP = COP_B - COP_T$$

The last quantity ($COP_T$) ranges from very low values around filtering areas of capillary wall to high values in regions where low capillary pressure has allowed reabsorption to take place. The total colloid os-

motic pressure of the blood is, in fact, the only force which is relatively constant, the other forces varying widely with circulatory adjustments. These variations, however, in general act between themselves to prevent excessive filtration of fluid through the capillary wall.

Whatever may be the importance of tissue pressure in limiting undue filtration under ordinary conditions, the mere existence of edema shows in itself that the power of the tissues to resist accumulation of tissue fluid is limited. Landerer (1884) believed that all forms of edema were to be explained by reduced elasticity of the tissues. In determining the modulus of normal and edematous tissues he failed to take in account that a given mass of edematous tissue contains fewer connective tissue fibers and more fluid than the same volume of normal tissue. It would be expected, therefore, that under a given force edematous tissue would stretch more easily. Bönniger (1905) tested the elasticity of the skin taken from cadavers and found no evidence that the elasticity of tissue was diminished in edema. In fact the skin removed from an edematous extremity shortened more than did skin taken from a normal extremity. Bönniger observed also that the tissues are very imperfectly elastic so that after stretching they fail to return immediately to their original dimensions though tension is removed. Therefore a relatively low tissue pressure acting for a long time may gradually stretch the tissue, the elasticity of which delays, but cannot prevent, the appearance of edema.

Additional evidence of the ease with which the tissues stretch was given by Iversen (1928) who found in studies on ascites that intraabdominal pressure is reduced almost to atmospheric pressure after relatively little fluid is removed. After stretching, the tissues of the abdominal wall are capable of accommodating a considerable volume of fluid without great increase in extravascular pressure.

Few measurements of tissue pressure in edema are available. The tightness of the skin in certain massive edemas suggests that the tissue pressure is quite high under certain conditions. Yet Holland and Meyer (1932) failed to find the slightest evidence of such an increase in edema. It is a clinical truism that certain forms of edema appear first in the loose tissues of the orbit and face, but the looseness of the tissues is not the primary cause for the appearance of the edema since the relaxed abdominal skin of multiparae is not usually edematous (Fishberg, 1930). If, however, physical conditions allow filtration to exceed absorption conspicuously over long periods of time the loose tissues will show pitting edema before the more compactly constructed tissues. Weech, Snelling and Goettsch (1933) observed that in experimental animals

nutritional edema is apt to be correlated with less reduction of plasma protein than is edema due to plasmapheresis. Change in the elasticity of the tissues is suggested in order to explain this difference.

*Summary.* Tissue pressure modifies the movement of fluid through the capillary wall and normally prevents excessive filtration. Direct measurements show little agreement concerning the absolute level of normal tissue pressure. Plethysmographic studies of filtration indicate that tissue pressure modifies the effect produced by a given difference between capillary pressure and colloid osmotic pressure, particularly when that difference acts over brief periods of time. Direct measurements of tissue pressure and clinical experience indicate that when the tendency toward excessive filtration persists over long periods of time, the importance of tissue pressure is much less, presumably because the tissues are imperfectly elastic.

6. *Other factors possibly influencing the movement of fluid through the capillary wall.* The preceding sections of this review deal primarily with certain effects which a few of the more thoroughly studied physical factors exert on the movement of fluid and dissolved substances through the capillary wall. Enough evidence has accumulated to indicate that normally the movement of fluid through the capillary wall depends fundamentally upon capillary blood pressure, the colloid osmotic pressure of the plasma proteins and the retention of proteins by the capillary wall. Abnormality in one factor must be balanced by compensatory changes in the others if a normal balance between filtration and reabsorption is to be maintained. This does not mean, however, that all or even most of the observed facts can be explained in terms of the few and relatively simple forces already considered. On the contrary, it is to be expected that other forces, both fundamental and conditioning, will be recognized as knowledge increases.

The relation between lymph flow and tissue function has for a long time directed attention to influences, chemical or physical, originating in the tissues. Tissues differ with reference to their normal water content; according to recent studies (Skelton, 1927) half the body water is found in muscle and one-fifth in skin. The tissues vary, too, in their rate of yielding water to the blood stream during dehydration or after hemorrhage, and in their rate of receiving water from the blood stream during hydremic plethora (Skelton, 1927; Adolph and Lepore, 1931). Though the liver and intestines respond most quickly, it is muscle, because of its greater mass, which gives up more total fluid than any other tissue during fluid deprivation and takes up by far the greater

part of water added to the blood stream (Skelton, 1927). The mean arterial blood pressure is the only factor measured which varies in parallel with this muscle hydration (Adolph and Lepore, 1931). Blood pressure is more important than blood dilution in regulating the water content of tissues since infusions of gelatin and acacia produce dilution of the blood persisting for a longer time than the variation in muscle water. During hemorrhage, also, the changes in tissue water are initiated by alteration of blood pressure. Clamping the dorsal aorta modifies muscle water in accord with the change of mean arterial blood pressure. Adolph and Lepore (1931) found that increasing mean arterial blood pressure by 20 per cent raises the water content of muscle between 1 and 2 per cent. They conclude that changes in capillary blood pressure which accompany sudden changes in blood volume are important elements in controlling the initial exchange of fluid between blood plasma and tissues.

The state of extravascular fluid is also intimately concerned with the presence of free and bound water in relation to the colloidal constituents of the tissues. Peters (1933) has discussed the nature of interstitial and serous fluids, concluding that the existence of lymph, a fluid similar to blood serum and different from intracellular fluid, shows the tissue spaces between the capillaries and lymphatics must contain some fluid similar to both serum and lymph. In addition edema fluid is for the most part situated in spaces which are histologically demonstrable between the connective tissue fibrillae. Slight pressure on the skin displaces this fluid and, after external pressure is removed, the depression in the skin disappears when the fluid filters back to its usual situation. Fluid does not flow from a needle inserted into a block of swollen gelatin but edema fluid flows freely from a needle inserted under the skin. Were the capillary filtrate removed from the circulating blood by swelling of tissue colloids its withdrawal from the tissues should be far more difficult. The gradual transition from normal amounts of tissue fluid, through stages of pre-edema to outspoken edema make it reasonable to infer that such fluid is nothing more than an augmentation of normal tissue fluid. Peters (1933) suggests that the accommodation of this undifferentiated capillary filtrate by tissue cells would disturb the intracellular electrolyte balance which is highly complex and presumably of functional significance. Recent evidence, too, agrees in showing that relatively little of the water in the living organism is closely bound to colloids.

The free water in blood is practically equal to the total water being only 2 per cent less (Hill, 1930). In frog's muscle very little bound

water is found (Hill, 1930) and in mammalian muscle at least 94 per cent of the total water is capable of diluting electrolytes (Moran, 1930). Hetherington (1931) concludes from vapor pressure measurements that practically all of the water in the body is free and available to take part in osmotic interchanges between blood, tissues and cells. Even colloidal solutions such as gelatin, gum acacia, casein or starch show little or no detectable hydration (Grollman, 1931; Greenberg and Greenberg, 1932).

According to Fischer (1921) swelling of tissues and edema is to be ascribed to the accumulation of acids resulting from disturbance of circulation or to local injury. This concept, based on the effects of large changes in hydrogen ion concentration on swelling of colloids outside the body, has been destructively criticized by Schade and Menschel (1922) and by Schade (1927). Lucké and McCutcheon (1926a, b) found that the unfertilized eggs of Arbacia do not swell in acid solutions except after injury or death. In general death is accompanied by irreversible gelation of the cells.

Connective tissue is in contact with both capillaries and body cells and, of all tissues, is most likely to be concerned with the movement of fluid through the capillary wall (Schade and Menschel, 1923). The amount of water in connective tissue depends upon mechanical pressure, hydrogen ion concentration, and salts, in order of importance. The collagenous fibers and the so-called ground substance, the two components of connective tissue, react differently to certain of these forces and the net change in tissue volume depends upon the algebraic sum of the effects on the two components. According to the summary given by Schade (1927) if pH changes from 7.35 to 5.90 the volume of connective tissue increases by 15 to 20 per cent. If the concentration of sodium chloride changes from 0.5 to 1.0 per cent swelling amounts to between 3 and 9 per cent. If all the connective tissue in the body were to swell 10 per cent, approximately one liter of water might be retained. This quantity is, however, relatively small compared to the volume of the extravascular fluid required to produce edema. Moreover to obtain even this slight change requires that the electrolyte balance shift well beyond the ordinary range of physiological variation.

Del Baere (1931) made incisions into the skin and determined the pressure required to balance what he termed the swelling pressure of the tissues. Since these observations apply to injured tissue in which fluid balance is influenced not only by leakage through the tissue spaces but also by filtration and reabsorption from presumably dilated and injured capillaries, it is doubtful whether the absolute figures are significant.

Meyer (1932) states that even the filtration produced by venous congestion should be ascribed to changes in the tissues rather than to distortion of the balance between capillary blood pressure and the colloid osmotic pressure of the blood. The rates of filtration observed by Landis (1927b), Krogh, Landis and Turner (1932) and Landis and Gibbon (1933) are directly proportional to capillary or venous pressure respectively. Moreover, independent changes in venous pressure and colloid osmotic pressure modify the filtration rate by similar amounts (Krogh, Landis and Turner, 1932). It would be an extraordinary coincidence if this relationship, which accords with physical principles, were due to some undefined change in the tissue associated with anoxemia or with the retention of metabolic products. Moreover White, Field and Drinker (1933) have shown that elevating venous pressure increases the flow of lymph from the congested region. If the change in water distribution were due to swelling of the tissues this fluid should not be free and would not flow from the part through the lymphatic vessels.

Aldrich and McClure (1923, 1924) found that wheals produced by the intradermal injection of physiological salt solution disappear more rapidly from edematous skin than from normal skin. This was taken to show that edematous tissues exhibit a heightened affinity for water. Salt solution in 20 per cent and 4 per cent strengths disappears with equal rapidity (Chevallier and Stiffel, 1925). Elevating the limb prolongs, and lowering the limb shortens, the time required for the wheals to disappear (Guggenheimer and Hirsch, 1926). Wheals of normal salt solution and of paraffin oil behave similarly with respect to their rates of disappearance from normal and edematous tissue (Govaerts and Bernard, 1927). Posture should not change the affinity of tissue for water. Paraffin cannot be bound by tissues nor can it be absorbed by the capillaries. Separation of the tissue elements in pre-edema or edema opens up channels in the tissues, and it is through these that salt solution or oil filters after intradermal injection. The rapid disappearance of cutaneous wheals in edema is therefore primarily mechanical in origin.

From the evidence at hand it is unlikely that binding of water by tissues plays an essential rôle in determining the distribution of fluid between blood and tissue spaces. Those effects which have been observed occur slowly and are too small to explain the modifications of fluid balance observed under physiological conditions. It is conceivable, however, that swelling of tissue colloids plays a rôle in the pathogenesis of certain rare intracellular edemas.

The stimulation or section of nerves modifies the rate at which fluid and dissolved substances pass through the capillary endothelium (Asher, 1922; Kajikawa, 1922; Yamamoto, 1924; Fleiderbaum, 1931; Hesselmann, 1932). Such procedures, however, alter capillary blood pressure, capillary diameter and blood flow and it is impossible to state whether the effects on diffusion, absorption and filtration are due to intrinsic change in capillary permeability or to changes in circulation. Since the latter are known to modify the interchange of substances between the blood and tissue spaces, it is essential that they be carefully controlled before a change in capillary permeability is postulated.

Certain hormones have been studied with reference to their effects on capillary permeability and fluid balance. Pituitrin, first studied by Krogh and Harrop (1921b) does not prevent edema from appearing in the frog's leg during perfusion (Drinker, 1927) nor does it keep protein from leaking through the mammalian capillary wall when the blood pressure is abnormally low (Blalock, Wilson, Weinstein and Beard, 1933). It does not prevent the local edema which follows the conjunctival installation of mustard oil (Saxl and Donath, 1925). The flow of lymph from the thoracic duct, however, is decreased after pituitrin injections (Bayley et al., 1925; Petersen and Hughes, 1925) and pitressin diminishes the flow from the leg lymphatics (Haynes, 1932b). In these last observations the capillary wall was normal and circulatory effects can explain the modification of lymph flow.

The usefulness of epinephrine and ephedrine administered hypodermically in the treatment of certain forms of localized clinical edema remains unexplained. According to Tainter (1926) the gross edema of paraphenylenediamine, which is due primarily to endothelial injury, can be prevented by the slow intravenous injection of epinephrine or by continued stimulation of the cervical sympathetic nerve. Moderately large and repeated doses of nicotine, strychnine, picrotoxin and santonin have the same effect, by increasing the output of epinephrine from the adrenal glands. It is suggested that edema is less owing to reduced blood flow incident to vasoconstriction. Thus the action of adrenalin in paraphenylenediamine edema may be due to a reduction of capillary blood pressure similar to the local action described by Hirschfelder (1924). Yet epinephrine added to cocaine and saligenin increases their tendency to cause local edema (Hirschfelder, Backe and Jennison, 1925). This effect of relatively large local dosage may possibly be attributed to injury of the endothelium from prolonged vasoconstriction and anoxemia. The observations of Freeman (1933) on vasoconstriction and

shock are relevant in this regard. More recently the hormones of the adrenal cortex have been shown in the intact animal to modify fluid balance after hemorrhage (Swingle et al., 1934) but the exact mechanism is not yet clear (Britton and Silvette, 1934). Previous thyroidectomy and hyperthyroidism modify the rate at which edema develops in the perfused surviving limbs of mammals (Sato, 1928a, b, c).

The effects of calcium on capillary permeability and fluid balance are difficult to interpret on account of the conflicting nature of results obtained under different circumstances. Earlier work on the effects of calcium was reviewed in detail by Loeb (1923) who concluded that its effect on endothelial permeability cannot be very striking. Hamburger (1922) observed that calcium contracts the vessels in a perfused limb, and that this vasoconstriction is released by potassium. Schmidt (1921), however, described dilatation and diminished sensitivity of vessels to adrenalin following the use of a perfusion fluid containing calcium. According to Lipschitz and Schmitt (1932) the subcutaneous administration of calcium chloride inhibits inflammatory reactions while intravenous injection of a similar amount has a very uncertain effect. The confusing results are in all probability due to varying dosage and circulatory effects.

*Summary.* The available evidence indicates that swelling of the tissues accounts for relatively little retention of fluid. Bound water is not present in sufficient quantity to modify the balance between the capillary blood and the tissue fluid appreciably. Extravascular fluid produced by the physical forces considered in previous sections rests in the interstitial spaces in free form. Innervation, hormones and calcium affect the movement of fluid and dissolved substances through the capillary wall, but their action cannot be ascribed definitely to changes in capillary permeability since the observations are complicated by possible modifications of blood flow and particularly of capillary blood pressure.

III. Complex changes in capillary pressure and permeability.

1. *Temperature.* Though fluid movement is conspicuously modified by heat or cold there is as yet no direct evidence that these changes are due to modification of capillary permeability *per se*, unless the change in temperature is great enough to injure the endothelium. Slight changes in capillary permeability due to temperature variations are by no means ruled out, but even if present they would have little effect on the concurrent processes of filtration and reabsorption since, all other factors remaining constant, more rapid filtration in one area would be balanced by more rapid reabsorption elsewhere. Only if capillary permeability is

increased to the point where protein passes with ease will absorption cease. Thus excessive heat injures the capillary wall and produces edema, but this is the usual effect of tissue damage and not a specific effect of temperature.

Bazett (1927) mentions the common knowledge that the digits swell during warm weather and shrink during cold weather. This change in volume is partly due to the amount of blood in the vessels though the rate of change is too slow to be explained satisfactorily on this basis alone. Local heat dilates the superficial vessels and blood flow is much increased. Average capillary pressure in the skin of the finger under normal conditions amounts to 32 mm. Hg in the arteriolar limb and 12 mm. in the venous limb. When skin temperature is elevated to 42°C. capillary pressure rises to 60 and to 45 mm. in the arteriolar and the venous limbs respectively (Landis, 1930b). The excess of capillary blood pressure over the colloid osmotic pressure of the blood must lead to the filtration of fluid which presumably passes on into the lymphatic vessels.

Local heat also increases the total filtering area by dilating capillaries previously closed or only partially open. Both factors or either factor singly, would tend to increase the rate of filtration at any given venous pressure. It is impossible to estimate the relative importance of these two possibilities from the data at present available.

Drury and Jones (1927) found with a simple plethysmograph that a given venous pressure applied when the foot was immersed in water at 42° filtered fluid from two to five times as rapidly as the same venous pressure at 16°. Landis and Gibbon (1933) with a pressure plethysmograph found that the filtration produced by venous pressures from 30 to 60 cm. water was twice as great at 45° as at 15°C.

These results have been interpreted (Bazett, 1932) by assuming a linear relationship between the rate of edema formation and the difference between capillary pressure and the osmotic pressure of the plasma proteins—a relationship demonstrated by experiment (Landis, 1927; Krogh, Landis and Turner, 1932; Landis and Gibbon, 1933). The results imply that with a sufficiently high local temperature filtration predominates, and continual lymphatic drainage takes place.

The general effects of temperature on fluid movement are less uniform and clear. Hamilton and Barbour (1925) studied the effect of cold on the transport of fluid from the blood to the tissue spaces. When anesthetized dogs were kept lying on slabs of ice for twenty to fifty minutes immediately before they were killed, more water was found in the subcutaneous tissue and muscle of the cooled side. Barbour and Hamilton

(1925) postulated, therefore, that exposure to cold produces anhydremia because fluid is diverted to the cooled skin, subcutaneous tissues and muscles as a result of arteriolar constriction, capillary dilatation from anoxemia, and increased permeability. In plethysmographic studies lowering the temperature of the forearm to 14.5° Centigrade did not increase tissue volume (Drury and Jones, 1927; Landis and Gibbon, 1933). Bazett (1927) points out that the generalization made by Barbour and Hamilton seems unwarranted for no control observations were made to determine the effect of gravity. Moreover, ice might well be an excessive stimulant producing the effects of slight frostbite rather than those of simple cold. Lewis (1930) postulated that the reactive dilatation which comes on when the skin is cooled below 15° Centigrade is due to an axone reflex stimulated by injury and the secondary release of H-substance in the cooled skin. Excessive cooling can produce blistering and all the evidences of local injury (Lewis and Love, 1926). The blister fluid obtained from these areas contains protein in a concentration approaching that of blood, indicating endothelial damage. Excessive cold, while diminishing capillary pressure temporarily, increases capillary pressure during the period of reaction (Landis, 1931).

Excessive heat leads to the formation of a blister with fluid rich in protein and produces the inflammatory changes secondary to tissue injury. The exudate contains certain substances arising from injury of tissue and the blister fluid when injected beneath normal skin produces there both vascular dilatation and increased capillary permeability (Lewis and Grant, 1924).

Heat increases the rate at which dyes pass through the capillary wall (Okuneff, 1924; Hudack and McMaster, 1932) though the staining of the tissues does not become uniform until the capillary wall is damaged (Hudack and McMaster, 1932). Injury due to heat increases lymph flow and the pressure in the lymphatic vessels may reach 120 cm. water (Field, Drinker and White, 1932). The protein content of such lymph approaches that of blood owing to endothelial injury.

The striking effect of lesser changes of temperature on the filtration produced by venous pressures of 30 cm. water or more is of particular interest with regard to the appearance, or the accentuation of certain forms of edema in response to climatic changes. Frequently patients with mild edema complain that the swelling of the lower extremities is greater during warm weather than during cold. Castellani (1931) in a recent review of minor tropical diseases mentions two types of heat edema. The milder form is "extremely common all over the tropics,

and Europeans on their way to the East frequently develop it as soon as the boat reaches the southern portion of the Red Sea and Aden." The feet and legs swell slightly and pitting occurs without any evidence of renal or cardiac abnormality. In more severe form heat edema was observed by Castellani in Europe and America during a heat wave. The edema comes on suddenly, lasts as long as the high temperature persists and then disappears. This type of edema involves the lower extremities, pits slightly on pressure and, like the first variety, is not accompanied by abnormal renal or cardiac function.

In a warm environment, according to Lewis and Pickering (1931) the cutaneous vessels of the upper extremities dilate in order to promote the loss of heat. Gibbon and Landis (1932) found that immersing the forearms in warm water produces in the lower extremities a maximal vasodilatation which is usually comparable in degree to that obtained by spinal anesthesia. During such vasodilatation the total area of capillary wall available for filtration in the extremities must be increased. In the erect posture venous pressure in the lower extremities reaches very high levels due to the hydrostatic pressure of the column of venous blood. Thus high venous pressure and greater filtering area favor the filtration of fluid. According to de Almeida (1920) high temperature increases the rapidity with which local edema is produced by venous congestion in the frog. Heat edemas may be at least partially due to physical effects similar to those exerted by local heat on the filtration of fluids through the normal capillary wall.

*Summary.* The effects of heat on fluid movement through the capillary wall are explicable on the basis of *a*, capillary dilatation which increases the area available for fluid movement; *b*, rise in capillary pressure which favors filtration, and *c*, injury by which heat, like other noxae, increases capillary permeability to colloids and consequently lowers the effective osmotic pressure of the plasma proteins. The effect of temperature on the permeability of the capillary wall to fluid alone has not been ruled out but, on theoretical grounds, other factors remaining constant, cannot be expected to influence conspicuously the balance between blood and tissue fluid.

2. *Tissue function in relation to capillary pressure and permeability.* The effects exerted on local blood flow and capillary diameter by products of metabolism have been reviewed in detail by Lewis (1927) and Krogh (1929). Secretory activity (Asher, 1908) and muscular contraction (Barcroft and Kato, 1915–16) are accompanied by a conspicuous increase of blood flow through the active tissues. Even temporary

anemia of the tissues produces reactive hyperemia (Lewis and Grant, 1924). The number of open capillaries in exercising muscle is much greater than in resting muscle (Krogh, 1919; Martin, Woolley and Miller, 1932) and the surface available for filtration is proportionately increased. Arteriolar dilatation and the opening of previously closed capillaries are generally ascribed to action of metabolic products (Asher and Barbera, 1898; Harris, 1922; Lewis and Grant, 1925). While there is not complete agreement concerning the specific metabolic product which produces vasodilatation (Krogh, 1929), the relation between tissue activity and hyperemia is not questioned.

Many observations have shown also, that tissue activity is accompanied by a shift in the equilibrium between the circulating blood and tissue fluid. It is an old observation that though lymph can be obtained only with difficulty from resting muscle, it flows freely during and after a series of contractions. Physiological activity increases the flow of lymph, usually but not always, from salivary glands (Asher and Barbera, 1898; Bainbridge, 1900; Moussu, 1900; Carlson, Greer and Becht, 1907); from the liver (Asher, 1899) and from the kidney (Schmidt and Hayman, 1929–30). Muscular activity also increases lymph flow. Barcroft and Kato (1915–16) compared the hemoglobin content of blood before and after its passage through contracting muscle and found that the blood loses fluid at the average rate of 1.0 cc. per hundred grams of tissue per minute. During exercise the muscles become heavier so that the rate of lymph flow must be something less than this. White, Field and Drinker (1933) cannulated the lymphatic vessels and found that muscular activity increases the flow of lymph but the lymph produced per gram of tissue cannot be computed from their data.

Capillary blood pressure rises following tetanus of muscle. In resting frog's muscle average arteriolar capillary pressure is 14.9 cm. water while average venous capillary pressure is 9.5 cm.; during the hyperemia which follows tetanus average arteriolar capillary pressure is 20.1 cm., and average venous capillary pressure 16.0 cm., without significant change in arterial or venous pressures (Landis, 1931). Pulse pressure in the capillary network is increased to several centimeters of water during this period of hyperemia. The conspicuous increase in capillary pressure must favor the movement of fluid toward the tissues.

It is unfortunate that there are no direct estimates of the amount of lymph produced per gram of active tissue. According to the indirect determinations of Barcroft and Kato (1915–16) filtration during muscular activity exceeds the possible filtration to be expected from a simple rise of

capillary blood pressure. Landis and Gibbon (1933) found in the human forearm that a venous pressure of 80 cm. of water causes fluid to accumulate in the tissue spaces at a rate of only 0.20 cc. per minute per hundred cc. of tissue, while Barcroft and Kato computed that in contracting muscle blood loses fluid at the rate of 2 cc. per minute per hundred grams of functioning tissue. Thus during muscular activity fluid leaves the blood at least several times more rapidly than it does under a venous pressure of 80 cm. water during rest. This suggests that some other factor is concerned in producing the high rate of lymph flow characteristic of functional activity of tissues.

Because of the close relation between tissue activity and lymph flow Asher and Barbera (1898) postulated that metabolic products must change the osmotic relationship between lymph and blood. Barcroft and Kato (1915–16) considered the formation of lymph in activity to be related to *a*, increased capillary pressure (following Starling, 1896) and increased metabolism (following Asher and Barbera, 1898). According to Barcroft and Kato the different points of view stressed by Starling and Asher, while frequently thought to be antagonistic, are really not in any true conflict. Metabolic products produce dilatation of the arterioles in active organs and cause local increase of capillary blood pressure with greater lymph flow. It is reasonable to suppose that functional activity, through the production of osmotically active substances, causes fluid to move toward the tissues by raising temporarily the osmotic pressure of the extravascular fluid, and possibly by increasing the permeability of the vessels. Barcroft and Kato were able in a few instances to show that if blood flow is constant and metabolism variable, the loss of fluid from the blood changes with metabolism. Given a constant metabolism, however, the loss of fluid from the blood changes with blood flow. They conclude that functional activity increases filtration *a*, directly, by altering the osmotic properties of the system, and *b*, indirectly, by inducing vasodilatation and increased capillary pressure.

This view explains why lymph flow during muscular activity exceeds the filtration produced during rest by a simple rise in capillary blood pressure. Muscular activity is accompanied by the production of osmotically active substances, which are diffusible and which, by reason of their smaller molecular size, have an osmotic pressure greater than the parent substances from which they were derived. According to Hill and Kupalov (1930) the frog's sartorius muscle, when completely fatigued, has an osmotic pressure greater than normal by an amount, which is equivalent to 0.35 per cent of NaCl solution. This, of course,

represents an extreme condition following anaerobic contraction. The change in the vapor pressure of the circulating blood during exercise shows that significant local changes in osmotic pressure in contracting muscle are at least within the range of possibility. According to Margaria (1930) severe muscular exercise raises the vapor pressure of human blood from the equivalent of 0.9447 gram of NaCl in 100 grams of water to the equivalent of 1.048 grams of NaCl in 100 grams of water.

Since these osmotically active substances diffuse from muscle into the extravascular fluids, and then into blood, it is logical to suppose that fluid will be withdrawn from the blood stream until diffusion restores the osmotic equilibrium which usually obtains between the diffusible constituents of blood and tissue fluid. No change in capillary permeability is required since this movement of fluid is due solely to temporary inequality of osmotic pressure inside and outside the capillary wall.

Shimizu (1931) observed in animals that muscular activity of mild grade had no uniform or conspicuous effect on the lactic acid content of blood. The amount of lactic acid in lymph, however, was always increased. He concludes that in dogs during muscular exercise, lactic acid is produced in the muscles, diffuses into the lymph spaces and finally reaches the circulation.

The movement of fluid toward functioning tissues might conceivably be concerned also with a change in the permeability of the capillary wall itself. This is suggested by the relatively high protein content of lymph collected from dogs during walking (White, Field and Drinker, 1933). According to Lazarus-Barlow (1894) simply stopping blood flow produces little transudation into the tissue spaces, but when accompanied by muscular activity it causes obvious edema. The lymph then becomes abnormally rich in protein, indicating that capillary permeability is increased.

Under normal conditions the glomerular filtrate produced in the kidney is nearly or quite free of protein (Wearn and Richards, 1924). Stoppage or even reduction of renal blood flow produces a transient albuminuria, which is not accompanied by any microscopically visible pathologic change (Starr, 1925a, b). Apparently temporary asphyxia increases the permeability of the glomerular tuft so that protein escapes from the plasma when blood flow is reëstablished.

Metabolism of adjacent tissues might modify endothelial permeability through 1, lowered oxygen tension; 2, increased carbon dioxide tension, or 3, local increase in acidity. From micro-injection experiments Landis (1928) concluded that, after three minutes' anoxemia, fluid filters through

the frog's capillary wall four times more rapidly than normal. After a period of oxygen lack the capillary wall, normally impermeable to protein, permits protein to pass and the effective osmotic pressure of the plasma proteins is reduced to one-half normal. The movement of fluid through the asphyxiated capillary wall is still directly proportional to the difference between capillary pressure and the effective osmotic pressure of the plasma proteins, indicating that the endothelium acts merely as a passive filter, though more permeable than normal. When blood flow is resumed the capillary wall rapidly recovers its impermeability to protein and the rate of fluid movement also returns practically to normal. This indicates that the effects of oxygen lack, if sufficiently brief in duration, are almost completely reversible.

Exposing the frog's mesentery to Ringer's fluid half saturated with carbon dioxide does not modify fluid movement; complete saturation increases the rate of fluid movement very slightly, but the wall still remains normally impermeable to protein. Increasing the hydrogen ion concentration within physiological limits produces almost no change in fluid movement until, at a pH of 4.0, the characteristic effects of injury appear.

Though brief, complete lack of oxygen increases capillary permeability conspicuously and reversibly it is still doubtful whether the oxygen tension in functioning tissues ever becomes low enough to be of importance. Anoxemia would have to occur in spite of increased blood flow and the opening of many new capillaries (Krogh, 1929). The possible effects of oxygen lack on capillary permeability must, however, be kept in mind on account of the evidence that the reactions of larger vessels (Adler, 1921) and of the capillaries themselves (Heimberger, 1926–27; Goldschmidt and McGlone, 1932) are modified by their oxygen supply.

There is little information concerning the importance of long-continued, mild oxygen lack. Bolton (1903–16) concluded that the edema of passive congestion, which follows ligation of the vena cava must be caused in large part by endothelial injury resulting from reduced blood flow, since in chronic experiments capillary blood pressure is not elevated except for a brief period immediately after the veins are ligated. In cardiac decompensation the edema fluid sometimes, but far from always, contains more protein than extravascular fluid rising from mechanical causes. This is possibly related also to capillary damage following long-continued, mild anoxemia.

Lymph collected from the subcutaneous lymphatics of walking dogs

contains from 1.52 to 0.5 per cent of protein (White, Field and Drinker, 1933). The protein content of this lymph is considerably greater than that ordinarily found in extravascular fluid or lymph produced by simply elevating capillary blood pressure. Moreover, lymph collected during exercise ordinarily contains red cells and this suggests also that the capillary wall is in some manner modified by muscular activity. Cohnheim (1867) observed in the frog's web that severe venous congestion produces capillary dilatation and, after a period of hours, extravasation of red cells, singly or in the form of small hemorrhages. Yet when venous congestion is relieved the closely packed cells within the capillaries are rapidly washed out and circulation continues quite normally in spite of the fact that orifices large enough to admit red cells had previously appeared in the capillary wall. Cohnheim expressed the belief that under pressure erythrocytes may escape through preformed stomata which close when pressure is reduced to normal. Turner (1933) observed that red cells escape sometimes directly into lymphatics, more often into the tissue spaces, during maximal venous obstruction combined with exercise. Drinker and Field (1933) found that venous pressure fluctuates during muscular activity; the pressure in the veins at the knee of the dog may be doubled when the leg muscles contract, falling instantly to the original value or lower when the muscles relax. The capillaries in the foot are subjected to a steady, high venous pressure when the individual stands quietly, and to a fluctuating pressure when he walks or runs. Microscopically it may be observed that circulation is much modified during tetanus of muscle when groups of capillaries are obviously distended, probably from pressure exerted by the muscle fibers on the small venules draining the region under observation (Landis, 1931). Blood will frequently spurt from such capillaries into the venules when the muscle relaxes.

If India ink be introduced under a pressure of 50 to 60 mm. Hg into single capillaries of the frog's mesentery some of the pigment escapes from weak spots in the capillary wall, collecting outside the vessel not uniformly, but in discrete collections. Yet afterward blood flows through such a vessel quite normally without undue loss of fluid, in spite of the fact that previously the wall had permitted the passage of India ink, to which even the damaged capillary wall is impermeable (Landis, 1934). Evidence is accumulating (Drinker, 1934) to show that activity may, in fact, produce temporary openings in the capillary wall through which erythrocytes may pass into the extravascular tissue. Apparently these

openings can disappear without modifying the normal permeability of the capillary wall under ordinary conditions since in some experiments mentioned by Drinker blood flow continues without microscopic evidence of any distinct rupture of the endothelium. The relation of capillary permeability to mild, long-continued oxygen lack and to these temporary openings will probably explain certain perplexing anomalies of fluid movement during tissue activity.

*Summary.* Functional activity of the tissues produces hyperemia and with it a rise in capillary blood pressure. The increased flow of lymph which accompanies tissue activity is probably too great to be explained on the basis of a simple rise in capillary pressure. It is likely that osmotically active substances produced in the course of tissue metabolism are concerned with this copious flow of lymph. The accumulation of carbon dioxide and changes in hydrogen ion concentration within physiological limits have little, if any, influence on capillary permeability, certainly not enough to lower the effective colloid osmotic pressure of the plasma proteins to any measurable extent. Lack of oxygen, if extreme, can produce a temporary increase of permeability which is great enough to reduce the effective colloid osmotic pressure of the blood to half its normal value. There is less information concerning the effects on capillary permeability of mild anoxemia persisting over long periods of time. Openings appearing temporarily in the capillary wall may explain certain anomalies of fluid movement observed during muscular activity.

3. *The effects of injury on capillary pressure and permeability.* The effectiveness with which the plasma proteins retain water within the capillaries depends upon the vitality of the endothelium. Injury increases capillary permeability and reduces correspondingly the effective colloid osmotic pressure of the blood. It is well known that tissue damage influences the movement of fluid through the capillary wall in a complex manner, depending, among other things, on the grade of damage and the concomitant changes in blood flow, capillary pressure and capillary permeability.

The injury that is produced when a single capillary is pierced by a micro-pipette is extremely slight, but nevertheless is often followed by measurable changes in blood flow and capillary pressure. While measuring capillary blood pressure in man Landis (1930b) observed that piercing the skin with a micro-pipette, 4 to 8 micra in diameter, usually produced slight dilatation of the nearest capillary loop, a local reaction previously described by Heimberger (1925). When capillary

pressure was then determined repeatedly at frequent intervals, the level of pressure in most instances remained unaltered, but in approximately one-third of the measurements it rose by 2 to 6, or even 16 mm. of Hg after the first reading, reaching a maximum during the first or second minute. The time relations of this brief increase in pressure are similar to those of the normal reflex flare to pin prick described by Cotton, Slade and Lewis (1916–17). Thus the minute trauma produced by piercing a single capillary loop sometimes stimulates the axone reflex, producing local vasodilatation and a transient rise in capillary blood pressure. The direct injury involved is so slight, however, that no local wheal or visible edema develops. Similar transient changes in capillary pressure were observed following the insertion of a micro-pipette into single capillaries of the frog's web (Landis, 1931), the maximum pressure in this situation being 0.5 to 4.5 cm. of water above the original readings. This circulatory reaction to minimal trauma illustrates the extraordinary sensitiveness of the peripheral circulation to what may be termed the slightest possible mechanical injury.

If a single capillary in the frog's mesentery be compressed gently by means of a minute glass rod the effect of local injury on the permeability of the endothelium becomes apparent (Landis, 1927a). Colloidal dye solutions pass freely through such an injured area long before they can be detected outside normal areas of endothelium in the same capillary. The colored fluid does not spurt from a single point as it should if the endothelium were ruptured, but filters out uniformly along the whole damaged section. India ink introduced intravenously flows past the normal section of capillary but the carbon particles accumulate in dense masses on the inner surface of the injured endothelium. When mechanical injury is severe blood corpuscles and ink are commingled in a solidly packed collection indicating that endothelial permeability is maximally increased and that whole plasma has filtered through the damaged section of capillary wall. In this instance the permeability of the capillary wall increases without change in diameter. The leakage of plasma and of dye-stained fluid, with consequent packing of corpuscles, is not produced by a rise in capillary blood pressure *per se* since fluid is retained in the undamaged portion of the same capillary. Hydrostatic pressure is the same in the injured and normal portions of a single capillary, but plasma leaks only from the injured segment of endothelium. Moreover, if blood pressure is elevated in a normal capillary the corpuscles move together as fluid is filtered from the trapped blood, but this filtration ceases when the proteins are concentrated sufficiently to balance

the higher capillary blood pressure (Landis, 1927a, b). If the endothelium retains protein the corpuscles are, therefore, never tightly packed since some plasma remains within the capillary. Thus mechanical injury of single capillaries evidently produces filtration of whole plasma primarily because of increased capillary permeability.

Injury which, instead of being limited to a minute area, affects large portions of the peripheral vascular system, initiates a series of events which are fundamentally similar in their development. The effects of widespread injury are more difficult to analyze, however, because of added factors such as capillary dilatation, rise of capillary blood pressure, and changes in blood flow. Small amounts of harmful substances introduced intravenously, or applied to a tissue externally, produce simply vasodilatation, increased rate of blood flow and usually a rise of capillary blood pressure. This was described in detail for urethane (Krogh and Harrop, 1921; Krogh, 1922; Landis, 1927a), and in part for formaldehyde (Jacobj, 1923). In acute experiments circulation continues and edema is not conspicuous. The reaction to more severe injury begins also with vasodilatation, ascribed to direct injury of the contractile elements of the capillary and arteriolar walls (Gelpke, 1921; Jacobj, 1923), and increased blood flow. This is followed more or less rapidly (depending on the grade of injury) by microscopically visible concentration of the red cells, slower flow, and finally complete stoppage of circulation due to complete loss of plasma through the damaged endothelium (Krogh and Harrop, 1921a; Gelpke, 1921; Jacobj, 1923; Herzog, 1925; Landis, 1927a).

The development of true stasis is the surest sign of increased capillary permeability (Krogh, 1922). The escape of plasma can be followed microscopically from the first visible concentration of cells through the period of sluggish flow when the viscosity of the concentrated blood impedes venous outflow, to the final stage when the capillaries are filled completely with solid cylinders of tightly packed cells. Stasis ordinarily begins in the venous capillaries but the column of packed and immobile cells grows longer as new erythrocytes are added to it on the arterial side until the entire capillary network, and the smaller arterioles likewise, are involved. The term stasis has been used by Elliott (1921), Lennartz (1921) and Stegemann (1924) merely to designate cessation of flow without reference either to escape of fluid or to change in the ratio of corpuscles to plasma. In experiments dealing with capillary permeability, however, Krogh's use of the word has been adopted by Herzog (1925), Florey (1926b) and Landis (1927a); in each case the important

feature is the concentration of the red cells to which stoppage of flow is secondary. To avoid confusion, it is in this latter sense that the word is used in this paper.

Capillaries injured to the point where stasis occurs permit proteins (Landis, 1927b), colloidal dyes (Krogh, 1922; Herzog, 1925; Landis, 1927b; Menkin and Menkin, 1930) and colloidal starch (Krogh, 1922) to pass but still retain India ink quantitatively (Krogh, 1922; Herzog, 1925; Landis, 1927a). Krogh (1922) placed the size of the pores in damaged endothelium between 5 and 200 millimicra. The increased endothelial permeability of injury reduces the effective colloid osmotic pressure of the plasma conspicuously and increases by five to seven times the rate at which fluid filters through the capillary wall under the influence of a given difference between the effective colloid osmotic pressure of the blood and hydrostatic pressure (Landis, 1927b). Stickiness of the endothelium accompanies injury (Krogh, 1922; Herzog, 1925; Landis, 1927a) and affects not only the distribution of erythrocytes but of platelets (Landis, 1927a) which at times form colorless thrombi large enough to stop blood flow.

Increased endothelial permeability, though often accompanied by marked dilatation (Krogh, 1929) occurs also in constricted capillaries when highly injurious substances are applied to the vessels (Ricker and Regendanz, 1921; Landis, 1927a). Stasis often appears and resolves spontaneously without measurable change in capillary diameter (Landis, 1927a; Florey, 1926b). It is therefore primarily injury, and not stretching of the wall, which increases capillary permeability. It is logical, however, to suppose that injury sufficient to devitalize the endothelium might also diminish or even abolish the power of the contractile elements to maintain normal capillary tone.

The increased transudation through the walls of injured capillaries, though fundamentally dependent on greater capillary permeability, is related also to capillary blood pressure. The rate at which blood plasma leaves the injured capillary is greater during cardiac systole, when capillary pressure is high, than during diastole, when capillary pressure is slightly lower (Landis, 1927a). If systemic blood pressure is low stasis is absent or develops very slowly. Moreover, the development of stasis may be delayed or stopped at any moment if capillary pressure is reduced by blocking the arteriole supplying the injured region. Stasis develops more rapidly in a capillary network with rapid blood flow where capillary pressure is high than in a capillary network showing sluggish flow (Landis, 1927a).

Capillary pressure changes conspicuously as stasis develops. If dilute urethane be applied to the mesentery of the frog the capillaries dilate, capillary pressure rises slightly during hyperemia, but stasis does not occur. If a more concentrated solution is applied blood flow, at first rapid, becomes gradually more and more sluggish. When the corpuscles begin to concentrate in the venous capillaries and venules, capillary blood pressure rises quite suddenly to levels approaching arteriolar pressure. This striking rise in capillary pressure is ascribed to blockage of the venous capillaries by abnormally viscous, concentrated blood (Landis, 1927a). The transudation of fluid is favored by the higher filtration pressure and stasis, once begun, advances very rapidly. When the entire capillary network is blocked by cells in stasis capillary pressure is again low because solid plugs of cells in the finer arterioles interpose a block to arterial pressure. Thus while high capillary pressure does not in itself produce stasis, it favors the rapid development of stasis when the endothelium is injured. Resolution of stasis is favored by the lower capillary pressure observed during the later stages of injury.

Stasis develops rapidly or slowly depending upon the nature and the concentration of the injurious substance. The intravenous injection into a frog of gold or platinum salts is followed within a few minutes by conspicuous arteriolar dilatation, the opening of many new capillaries, and striking capillary dilatation. In a few minutes more capillary blood flow becomes slow, the corpuscles are concentrated and stasis develops in the turgid capillary network (Heubner, 1907; Gelpke, 1921). Eventually the capillary wall loses its continuity so that large or small hemorrhages appear. The whole series of events requires but 5 or 6 minutes and the more rapid the advance of stasis, the less likely is one to observe the original vasodilatation and the increased blood flow. Generally speaking, hyperemia with rapid blood flow is physiological and easily reversible; hyperemia with slow blood flow is pathological and return to normal conditions is difficult or even impossible.

If injurious chemical substances are applied externally in different concentrations to the frog's tongue or web the various stages of the reaction to external chemical damage may be dissociated. Urethane in dilute solution produces merely vasodilatation, but in concentrations toxic to other living cells also increases capillary permeability conspicuously (Landis, 1927a). According to Jacobj (1923) formaldehyde applied to the frog's web in concentrations less than 0.05 per cent does not change the peripheral circulation. An 0.05 per cent solution produces slight constriction which soon gives way to dilatation; 0.25 per

cent produces only dilatation and increased blood flow without any evidence of stasis. Stronger solutions after the period of rapid flow produce progressive slowing of flow with eventual stoppage in stasis. Formaldehyde in 1, 4, 16, 30 and 40 per cent strengths produces conspicuous stasis in 60, 55, 37, 18 and 3 minutes respectively. In each concentration the first effect is dilatation of all the vessels large and small. Jacobj ascribes this dilatation to paralysis of the contractile elements since they fail to react to sympathetic stimulation. Paraformaldehyde induces vasodilatation and increased blood flow continuing for a longer time; stasis appears only after some hours. The intravascular collections of cells then consist of both leucocytes and erythrocytes in the form of mixed thrombi.

If the injury is great enough to produce early stasis edema rarely appears since blood flow ceases in the injured area before sufficient fluid can pass out of the blood vessels to distend the tissues grossly (Ricker and Regendanz, 1921). Milder grades of damage persisting over longer periods of time, however, permit fluid to filter in sufficient amount to produce edema. Tainter and Hanzlik (1924) attribute the edema produced by paraphenylenediamine to injury of the capillary wall with escape of plasma protein. Colloidal dyes such as Congo red and trypan blue pass readily into the tissue fluid of the edematous region. The tissue fluid itself contains fibrinogen and forms a gelatinous coagulum in the distended tissue spaces. At blood pressures below 75 mm. Hg edema does not appear though at pressures above 85 mm. Hg the same dose of paraphenylenediamine produces edema regularly. This form of edema has no relation to colloid imbibition, local acidosis or to the central or peripheral nervous system. Local anesthesia or atropine has no effect on the appearance of the edema. The absence of a deep fascia in the head and neck favors the collection of edema fluid in these regions but edema can also be produced in the limbs of cats and rabbits (Gibbs, 1931). Edema of the lung does not appear, either because there is little or no normal interchange of fluid in this area (Gibbs, 1931), or because of the lower blood pressure in the pulmonary circuit.

Uranium salts, frequently used to produce experimental nephritis, also produce edema of the tissues if sufficient water is given the animal (Aubel, Mauriac and Boutiron, 1926) or if the tissues are perfused (Sato, 1928d). On account of the high protein content of the extravascular fluid Govaerts (1928a) concludes that the edema of acute uranium poisoning is primarily extrarenal in origin. Whitney (1928) found that uranium poisoning diminishes the rate at which antibodies penetrate

into the tissues and lymph; the reason for this slower penetration is not clear.

The wheal, a form of local edema, forms one part of the triple response reviewed by Lewis (1927) and Krogh (1929). Such localized collections of extravascular fluid can be produced in the skins of all young subjects when suitable stimuli are applied, though the susceptibility of the skin in different individuals varies (Lewis, 1924). Wheal fluid contains large amounts of protein (Lewis, 1924), and dyes which pass through the normal capillary wall very slowly, appear quickly in the wheals produced by stroking the skin (Hoff, 1927) or by injecting various irritant substances (Adlersberg and Perutz, 1932). External pressures above 30 to 50 mm. Hg prevent the wheals from forming even though the tissues have been injured. Lewis (1924) concludes that the wheal is due primarily to increased capillary permeability which is independent of capillary dilatation. The filtration of fluid and protein is not inherently due to increased filtration pressure, though aided considerably by high filtration pressure and rapid blood flow. Török, Rajka and Wessely (1928) found that mild venous congestion makes the wheal larger owing to high capillary pressure while severe venous congestion reduces its size owing to restricted blood flow. Starr (1930) uses the size of the histamin wheal to detect clinically the degree to which the flow of blood is reduced in peripheral vascular disease.

When once a wheal has been produced, a second stimulus in the same area after the disappearance of the first wheal fails to produce the gross evidences of increased capillary permeability in that area. Lewis and Grant (1924) attribute this to refractoriness, caused by alteration of the capillary endothelium so that it becomes for a time impermeable, even when stimulated and dilated a second time by histamin or a histamin-like substance. Hoff (1927) studied areas previously the site of whealing and, by elastometry, identified a latent edema which persisted after the wheal had to all external appearances gone away completely. According to Hoff (1927) in such a refractory area plasma and Congo red leave the blood stream rapidly but the skin is not elevated since the fluid leaks away from the injured area into the subcutaneous tissues through numerous fissures opened up by the previous edema in that region. The observations of Hoff are of considerable interest in connection with the rapid disappearance of intradermally injected salt solution from the skin of patients with edema or pre-edema. Pilcher (1926) observed that edema, whatever its cause, lessens wheal formation though in nephritis and heart disease unaccompanied by edema, the wheals produced by codein are normal.

Edema fluid removed from wheals in a case of Quincke's edema contained 3.12 per cent of protein, of which two-thirds was albumin with a trace of fibrinogen. Govaerts (1928b) concludes, therefore, that injury of the capillary wall, local in distribution, and of unknown origin, is responsible for this type of edema.

The local application of irritants to epithelial surfaces produces edema in the underlying tissue. Hirschfelder (1924) applied mustard oil to the conjunctiva of the rabbit and showed that this form of inflammatory edema is associated with demonstrable injury of the capillary wall. The edema is not due to imbibition of water by colloids, nor in any direct way to the activity of nerves. A low systemic blood pressure, or local vasoconstriction inhibits the appearance of edema. Vasodilator drugs increase the edema unless systemic blood pressure is very low. Local anesthetics and calcium chloride affect the edema in general accordance with their effect on local capillary blood pressure. Hirschfelder concludes that though the edema of mustard oil conjunctivitis is due primarily to injury of the capillary wall, the appearance of edema is conditioned by the capillary blood pressure in the inflamed area.

Local injury, chemical or thermal, if very severe often produces constriction of the arteries with simultaneous stasis in the capillary network (Ricker and Regendanz, 1921; Krogh, 1922; Landis, 1927a, 1931). If injury is mild, however, arteriolar dilatation and increased rate of blood flow are the rule, though prolonged action of mild noxae produces eventually the same result as a brief exposure to severe noxae. Ricker and Regendanz (1921) believe that the effects of injury, including even stasis, are due to stimulation of vasoconstrictor and vasodilator nerves, capillary permeability being affected secondarily. In some instances certainly (Landis, 1927a; 1928) injury affects the endothelium directly. Takenaga (1925) observed that the local redness produced by mustard oil appears though the sensory nerves to the inflamed area have degenerated. He concludes that the contractile elements of the capillaries are damaged by direct action, the axone reflex if involved being of secondary importance.

Heat and cold if sufficiently severe lead to blister formation (Lewis and Grant, 1924; Lewis, 1927); the fluid of the blister contains considerable protein indicating conspicuous damage of the capillary wall. Milder forms of heat and cold produce swelling of the skin without blisters, the swelling being due to an increase in the amount of subcutaneous fluid. Light, if sufficiently intense, injures the capillary wall and increases its permeability (Campbell and Hill, 1924; Levy, 1929; Hudack and McMaster, 1932).

Few measurements of capillary blood pressure have been made in inflamed tissues. Landis (1930) observed that freezing and the application of cantharides elevate cutaneous capillary blood pressure conspicuously. This must favor exudation after injury particularly since the effective colloid osmotic pressure of the blood is simultaneously reduced.

The systemic effects of endothelial injury and increased capillary permeability on blood volume have been studied exhaustively in relation to shock and need not be considered here. Recently Blalock, Beard and Thuss (1932) found that in animals whose blood pressure remained normal, salt, glucose or acacia solutions can be injected intravenously without diminishing the total circulating plasma protein. The fluid filtering through the capillary wall after salt and glucose injections is practically protein-free. In the presence of a declining blood pressure (Blalock, Beard and Thuss, 1932), local trauma (Beard and Blalock, 1932), and histamin (Beard, Wilson, Weinstein and Blalock, 1932), the circulating plasma protein diminishes considerably after similar intravenous injections indicating that plasma protein is lost through the capillary wall. Freeman (1933) injected adrenalin constantly over a period of hours in physiological amounts, finding that blood pressure and blood volume gradually diminish. He suggests that prolonged vasoconstriction of itself produces loss of fluid from the circulation. This is due presumably to increased capillary permeability from asphyxia after prolonged periods of diminished blood flow.

The inflammations seen in pathological conditions include in their early stages all of the reactions characteristic of local injury, but in later stages are far more complicated. There can be little doubt that the permeability of the capillary endothelium is increased in inflammation; colloidal dyes pass more easily (Hoff and Leuwer, 1926) and exudates, subcutaneous and serous, contain protein in concentrations approaching that in blood plasma. Even particulate matter passes through the capillary wall during true inflammation (Menkin, 1931b). Vasodilatation, increased blood flow, later sluggish blood flow and even stasis in isolated areas, with accompanying changes in capillary blood pressure are all found in various stages of inflammation.

The absorption of dyes or toxic substances injected into an injured tissue is often much delayed (Menkin, 1929; Underhill, Kapsinow and Fisk, 1930; Okuneff, 1930), and considerable evidence indicates that foreign substances in general are fixed in injured tissues (Menkin, 1931a, 1933) though lymph flows copiously from the same region (White, Field

and Drinker, 1933). This mechanism of nonspecific fixation protects the organism at the expense of local injury (Menkin, 1931a, c). Superimposed on the immediate effects of injury are leucocytic infiltration and the other reactions to long-continued, low-grade irritation. Schade (1927) reviews the changes in the tissues which play a rôle during inflammation by favoring the movement of fluid from the blood to the tissue spaces. The modified elasticity of connective tissue (Landerer, 1884; Schade, 1926), the greater depression of freezing point in purulent exudates (Schade, et al., 1926), modified reactions of blood vessels (Ricker and Regendanz, 1921; Krawkow, 1924), and possible increased metabolism of the injured tissues (Gessler, 1931a, b) all imply that inflammation, in both its development and its resolution, involves much more than the relatively simple changes in permeability and pressure which are characteristic of acute chemical or physical injury.

*Summary.* Local injury initiates a complex response including vasodilatation, rise in capillary blood pressure, first increased, then decreased blood flow, increased endothelial permeability and finally stasis. All forms of local or general edema produced by injury are due fundamentally to increased capillary permeability with easy passage of proteins and water through the endothelium. Agents which reduce systemic blood pressure or local capillary pressure prevent edema fluid from collecting even though the capillary wall is severely damaged.

## CONCLUSION

The movement of fluid through the capillary wall depends primarily upon the balance between capillary blood pressure and the colloid osmotic pressure of the blood. The effectiveness of this balance is modified, however, by endothelial damage, by tissue pressure, by temperature, by the accumulation of metabolic products and by other unidentified factors. Conclusions concerning capillary permeability are often made without considering in detail the many simple physical forces concerned in the movement of water and of dissolved substances through the capillary wall. Of these forces, capillary blood pressure is neglected more frequently than any other. This has resulted in the postulation of theories involving either active intervention of the endothelium or various modifications of its permeability under conditions inadequately controlled from the physical standpoint. Many facts concerning fluid balance, particularly in the intact animal or in patients with clinical edema, cannot be explained in terms of the simple physical forces which have been considered in this review. It seems probable, however, that adequate control of

these elementary forces will make the search for other factors both simpler and more productive.

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